Interview Summary	**Application No.** 09/497,891	**Applicant(s)** KUENZER ET AL.	
	Examiner Sabiha Qazi	**Art Unit** 1616	

All participants (applicant, applicant's representative, PTO personnel):

(1) *Sabiha Qazi, Ph.D*. (3)_____.

(2) *John A. Sopp (Attorney)*. (4)_____.

Date of Interview: *13 May 2005*.

Type: a)☒ Telephonic b)☐ Video Conference
c)☐ Personal [copy given to: 1)☐ applicant 2)☐ applicant's representative]

Exhibit shown or demonstration conducted: d)☐ Yes e)☐ No.
If Yes, brief description: _____.

Claim(s) discussed: *53-65*.

Identification of prior art discussed: _____.

Agreement with respect to the claims f)☐ was reached. g)☒ was not reached. h)☐ N/A.

Substance of Interview including description of the general nature of what was agreed to if an agreement was reached, or any other comments: *Examiner had called and discussed about the possiblity of allowable subject matter by amending the claims. Examiner told Mr. Sopp that if H as substituent at R17 and the compounds related to this amendment deleted than claims would be allowed. There will be no "new matter" situatiion. Mr. Sopp told the Examiner that the applicants would like to send to Board and will not amend the claims.* .

(A fuller description, if necessary, and a copy of the amendments which the examiner agreed would render the claims allowable, if available, must be attached. Also, where no copy of the amendments that would render the claims allowable is available, a summary thereof must be attached.)

THE FORMAL WRITTEN REPLY TO THE LAST OFFICE ACTION MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW. (See MPEP Section 713.04). If a reply to the last Office action has already been filed, APPLICANT IS GIVEN A NON-EXTENDABLE PERIOD OF THE LONGER OF ONE MONTH OR THIRTY DAYS FROM THIS INTERVIEW DATE, OR THE MAILING DATE OF THIS INTERVIEW SUMMARY FORM, WHICHEVER IS LATER, TO FILE A STATEMENT OF THE SUBSTANCE OF THE INTERVIEW. See Summary of Record of Interview requirements on reverse side or on attached sheet.

Examiner Note: You must sign this form unless it is an Attachment to a signed Office action.

____
Examiner's signature, if required

Summary of Record of Interview Requirements

Manual of Patent Examining Procedure (MPEP), Section 713.04, Substance of Interview Must be Made of Record
A complete written statement as to the substance of any face-to-face, video conference, or telephone interview with regard to an application must be made of record in the application whether or not an agreement with the examiner was reached at the interview.

Title 37 Code of Federal Regulations (CFR) § 1.133 Interviews
Paragraph (b)
In every instance where reconsideration is requested in view of an interview with an examiner, a complete written statement of the reasons presented at the interview as warranting favorable action must be filed by the applicant. An interview does not remove the necessity for reply to Office action as specified in §§ 1.111, 1.135. (35 U.S.C. 132)

37 CFR §1.2 Business to be transacted in writing.
All business with the Patent or Trademark Office should be transacted in writing. The personal attendance of applicants or their attorneys or agents at the Patent and Trademark Office is unnecessary. The action of the Patent and Trademark Office will be based exclusively on the written record in the Office. No attention will be paid to any alleged oral promise, stipulation, or understanding in relation to which there is disagreement or doubt.

The action of the Patent and Trademark Office cannot be based exclusively on the written record in the Office if that record is itself incomplete through the failure to record the substance of interviews.

It is the responsibility of the applicant or the attorney or agent to make the substance of an interview of record in the application file, unless the examiner indicates he or she will do so. It is the examiner's responsibility to see that such a record is made and to correct material inaccuracies which bear directly on the question of patentability.

Examiners must complete an Interview Summary Form for each interview held where a matter of substance has been discussed during the interview by checking the appropriate boxes and filling in the blanks. Discussions regarding only procedural matters, directed solely to restriction requirements for which interview recordation is otherwise provided for in Section 812.01 of the Manual of Patent Examining Procedure, or pointing out typographical errors or unreadable script in Office actions or the like, are excluded from the interview recordation procedures below. Where the substance of an interview is completely recorded in an Examiners Amendment, no separate Interview Summary Record is required.

The Interview Summary Form shall be given an appropriate Paper No., placed in the right hand portion of the file, and listed on the "Contents" section of the file wrapper. In a personal interview, a duplicate of the Form is given to the applicant (or attorney or agent) at the conclusion of the interview. In the case of a telephone or video-conference interview, the copy is mailed to the applicant's correspondence address either with or prior to the next official communication. If additional correspondence from the examiner is not likely before an allowance or if other circumstances dictate, the Form should be mailed promptly after the interview rather than with the next official communication.

The Form provides for recordation of the following information:
- Application Number (Series Code and Serial Number)
- Name of applicant
- Name of examiner
- Date of interview
- Type of interview (telephonic, video-conference, or personal)
- Name of participant(s) (applicant, attorney or agent, examiner, other PTO personnel, etc.)
- An indication whether or not an exhibit was shown or a demonstration conducted
- An identification of the specific prior art discussed
- An indication whether an agreement was reached and if so, a description of the general nature of the agreement (may be by attachment of a copy of amendments or claims agreed as being allowable). Note: Agreement as to allowability is tentative and does not restrict further action by the examiner to the contrary.
- The signature of the examiner who conducted the interview (if Form is not an attachment to a signed Office action)

It is desirable that the examiner orally remind the applicant of his or her obligation to record the substance of the interview of each case. It should be noted, however, that the Interview Summary Form will not normally be considered a complete and proper recordation of the interview unless it includes, or is supplemented by the applicant or the examiner to include, all of the applicable items required below concerning the substance of the interview.

A complete and proper recordation of the substance of any interview should include at least the following applicable items:
1) A brief description of the nature of any exhibit shown or any demonstration conducted,
2) an identification of the claims discussed,
3) an identification of the specific prior art discussed,
4) an identification of the principal proposed amendments of a substantive nature discussed, unless these are already described on the Interview Summary Form completed by the Examiner,
5) a brief identification of the general thrust of the principal arguments presented to the examiner,
   (The identification of arguments need not be lengthy or elaborate. A verbatim or highly detailed description of the arguments is not required. The identification of the arguments is sufficient if the general nature or thrust of the principal arguments made to the examiner can be understood in the context of the application file. Of course, the applicant may desire to emphasize and fully describe those arguments which he or she feels were or might be persuasive to the examiner.)
6) a general indication of any other pertinent matters discussed, and
7) if appropriate, the general results or outcome of the interview unless already described in the Interview Summary Form completed by the examiner.

Examiners are expected to carefully review the applicant's record of the substance of an interview. If the record is not complete and accurate, the examiner will give the applicant an extendable one month time period to correct the record.

Examiner to Check for Accuracy

If the claims are allowable for other reasons of record, the examiner should send a letter setting forth the examiner's version of the statement attributed to him or her. If the record is complete and accurate, the examiner should place the indication, "Interview Record OK" on the paper recording the substance of the interview along with the date and the examiner's initials.

In re Johnson and Farnham, 194 USPQ 187 (CCPA 1977)

In re Johnson and Farnham

(CCPA)
194 USPQ 187

Decided June 16, 1977
No. 76-643
U.S. Court of Customs and Patent Appeals

Headnotes

PATENTS

1. Claims -- Indefinite -- In general (§ 20.551)

Construction of specification and claims -- By prior art (§ 22.20)

Analysis of 35 U.S.C. 112 second paragraph rejection should begin with determination of whether claims satisfy requirements of second paragraph; first inquiry, therefore, is to determine whether claims set out and circumscribe particular area with reasonable degree of precision and particularity; it is here where definiteness of language employed must be analyzed, not in vacuum, but always in light of teachings of prior art and of particular application disclosure as it would be interpreted by one possessing ordinary level of skill in pertinent art.

2. Claims -- Indefinite -- In general (§ 20.551)

Claims -- Specification must support (§ 20.85)

Undue breadth of claims is not indefiniteness.

3. Construction of specification and claims -- By specification and drawings -- In general (§ 22.251)

Claim language must be read in light of specification as it would be interpreted by one of ordinary skill in art.

4. Claims -- Indefinite -- In general (§ 20.551)

Claims -- Specification must support (§ 20.85)

Pleading and practice in Patent Office -- Rejections (§ 54.7)

Specification -- Sufficiency of disclosure (§ 62.7)

Examiner's rejection premised on general ground that claims are "broader than the express limitation disclosed as defining the invention" and specific grounds that "express disclosure is clearly limited to the sigma value recited in claim 1," raises lack of enablement issue properly arising under first not second paragraph of Section 112.

5. Specification -- In general (§ 62.1)

Specification -- Claims as disclosure (§ 62.3)

It is function of specification, not claims, to set forth "practical limits of operation" of invention; one does not look to claims to find out how to practice invention they define, but to specification.

6. Claims -- Specification must support (§ 20.85)

Construction of specification and claims -- In general (§ 22.01)

Specification -- Sufficiency of disclosure (§ 62.7)

Specification as whole must be considered in determining whether scope of enablement provided by specification is commensurate with scope of claims.

7. Construction of specification and claims -- Broad or narrow -- In general (§ 22.101)

Patent grant -- Intent of patent laws (§ 50.15)

Specification -- Sufficiency of disclosure (§ 62.7)

Claims must adequately protect inventors to provide effective incentives; to demand that first to disclose shall limit his claims to what he has found will work or to materials that meet guidelines specified for "preferred" materials in involved process would not serve constitutional purpose of promoting progress in useful arts.

8. Applications for patent -- Continuing (§ 15.3)

Applicants are entitled to benefit of filing date of parent application that discloses invention of application in manner provided by Section 112, paragraph 1.

9. Claims -- Broad or narrow -- In general (§ 20.201)

Estoppel -- Involving interference (§ 35.20)

It is for inventor to decide what bounds of protection he will seek; it is applicant's right to retreat to otherwise patentable species merely because he erroneously thought he was first with genus when he filed.

10. Specification -- Sufficiency of disclosure (§ 62.7)

Notion that one who fully discloses, and teaches those skilled in art how to make and

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use genus and numerous species has failed to disclose and teach those skilled in art how to make and use genus minus two species and has thus failed to satisfy Section 112 first paragraph requirement results from hypertechnical application of legalistic prose relating to that provision of statute.

11. Pleading and practice in Patent Office -- In general (§ 54.1)

Specification -- Sufficiency of disclosure (§ 62.7)

While insufficiency under 35 U.S.C. 112 cannot be cured by citing causes for insufficiency, it is not true that factual context out of which question under Section 112 arises is immaterial; specification having described whole invention necessarily described part remaining after invention of another was excised.

Particular patents -- Polyarylene Polyethers

Johnson and Farnham, Polyarylene Polyethers, rejection of claims 1-9, 64, and 68-72 reversed.

Case History and Disposition:

Appeal from Patent and Trademark Office Board of Appeals.

Application for patent of Robert N. Johnson and Alford G. Farnham, Serial No. 230,091, filed Feb. 28, 1972, continuation-in-part of application Serial No. 295,519, filed July 16, 1963. From decision rejecting claims 1-9, 64, and 68-72, applicants appeal. Reversed; Lane, Judge, dissenting in part with opinion.

Attorneys:
Robert C. Brown and Aldo J. Cozzi, both of New York, N.Y. (James C. Arvantes, New York, N.Y., of counsel) for appellants.

Joseph F. Nakamura (Henry W. Tarring, II, of counsel) for Commissioner of Patents and Trademarks.

Judge:

Before Markey, Chief Judge, and Rich, Baldwin, Lane, and Miller, Associate Judges.

Opinion Text

Opinion By:
Markey, Chief Judge.

This appeal is from the decision of the Patent and Trademark Office (PTO) Board of Appeals affirming the rejection under 35 USC 102 or 103 (the rejection also raises a written description issue under 35 USC 112, first paragraph) of claims 1-9, 64, and 68-70 and the rejection under 35 USC 112, first paragraph (enablement) and second paragraph (indefiniteness), of claims 64 and 68-72 in appellants' application No. 230,091 filed February 28, 1972 (the 1972 application) for "Polyarylene Polyethers." [1] The 1972 application is a continuation-in-part of three earlier applications, the earliest being application No. 295,519 filed July 16, 1963 (the 1963 application). We reverse.

The Invention

The invention is in the field of polymer chemistry and more specifically relates to linear thermoplastic polyarylene polyether polymers composed of recurring units having the general formula
Graphic material consisting of a chemical formula or diagram set at this point is not available. See text in hard copy or call BNA PLUS at 1-800-452-7773 or 202-452-4323.
where O represents an oxygen atom, [2] E represents the residuum of a dihydric phenol [3] compound, and E' represents the residuum of a benzenoid compound having one or more inert electron withdrawing groups [4] in the ortho [5] or para [6] positions to the valence bonds and where both E and E' are bonded to the ether oxygens through aromatic carbon atoms.

Appellants describe a method of synthesizing these polymers by reacting a double alkali metal salt of a dihydric phenol with a dihalobenzenoid compound in the presence of certain solvents under substantially anhydrous reaction conditions.

The 1972 application includes the following disclosure with respect to the electron withdrawing group found in E' and in the E' precursor compound, that is, in the compound which is the predecessor of E' in the above general formula (we have designated paragraphs [A] and [B] and have added emphasis thereto):

> Any electron withdrawing group can be employed as the activator group in these compounds. It should be, of course, inert to the reaction, but otherwise its structure is not critical. Preferred are the strong activating groups such as the sulfone group
>
> Page 189
>
> *Graphic material consisting of a chemical formula or diagram set at this point is not available. See text in hard copy or call BNA PLUS at 1-800-452-7773 or 202-452-4323.*
> bonding two halogen substituted benzenoid nuclei as in the 4,4'-dichlorodiphenyl sulfone and 4,4'-difluorodiphenyl sulfone, although such other strong withdrawing groups hereinafter mentioned can also be used with equal ease.

The more powerful of the electron withdrawing groups give the fastest reactions and hence are preferred. It is further preferred that the ring contain no electron supplying groups on the same benzenoid nucleus as the halogen; however, the presence of other groups on the nucleus or in the residuum of the compound can be tolerated. Preferably, all of the substituents on the benzenoid nucleus

are either hydrogen (zero electron withdrawing), or other groups having a positive sigma a1value, as set forth in J.F. Bunnett in Chem. Rev. 49 273 (1951) and Quart. Rev., 12, 1 (1958). See also Taft, Steric Effects in Organic Chemistry, John Wiley & Sons (1956), chapter 13; Chem. Rev., 53, 222; JACS, 74, 3120; and JACS, 75, 4231. [7]

The electron withdrawing group of the dihalobenzenoid compound can function either through the resonance of the aromatic ring, as indicated by those groups having a high sigma a2value, i.e., above about +0.7 or by induction as in perfluoro compounds and like electron sinks.

[A]

Preferably the activating group should have a high sigma a3value, preferably above 1.0, although sufficient activity to promote the reaction is evidenced in those groups having a sigma value above 0.7, although the reaction rate with such a low powered electron withdrawing group may be somewhat low.

The activating group can be basically either of two types:

(a) monovalent groups that activate one or more halogens on the same ring as a nitro group, phenylsulfone, or alkylsulfone, cyano, trifluoromethyl, nitroso, and hetero nitrogen as in pyridine.

(b) divalent group [sic] which can activate displacement of halogens on two different rings, such as the sulfone group
Graphic material consisting of a chemical formula or diagram set at this point is not available. See text in hard copy or call BNA PLUS at 1-800-452-7773 or 202-452-4323.
; the carbonyl group
Graphic material consisting of a chemical formula or diagram set at this point is not available. See text in hard copy or call BNA PLUS at 1-800-452-7773 or 202-452-4323.
; the vinyl group
Graphic material consisting of a chemical formula or diagram set at this point is not available. See text in hard copy or call BNA PLUS at 1-800-452-7773 or 202-452-4323.
; the sulfoxide group
Graphic material consisting of a chemical formula or diagram set at this point is not available. See text in hard copy or call BNA PLUS at 1-800-452-7773 or 202-452-4323.
; the azo group -N=N-; the saturated fluorocarbon groups $-CF_2CF_2-$; organic phosphine oxides
Graphic material consisting of a chemical formula or diagram set at this point is not available. See text in hard copy or call BNA PLUS at 1-800-452-7773 or 202-452-4323.
; where R is a hydrocarbon group, and the ethylidene group
Graphic material consisting of a chemical formula or diagram set at this point is not available. See text in hard copy or call BNA PLUS at 1-800-452-7773 or 202-452-4323.
where X can be hydrogen or halogen or which can activate halogens on the same ring such as with difluorobenzoquinone, 1,4- or 1,5- or 1,8- difluoroanthraquinone.

[B]

Those skilled in the art will understand that a plurality of electron withdrawing groups may be employed if desired, including electron withdrawing groups having a sigma a4value below about +0.7 provided the cumulative sigma a5influence on each of the reactive halogen groups of the halobenzenoid compound is at least about +0.7.

The Disclosure and Prosecution History of the 1963 Application

To understand the written description issue in this appeal, it is necessary to summarize the disclosure and prosecution history of the 1963 application. The 1963 application described (and claimed) in haec verba a genus of polymers as defined by the above general formula. That application stated:

> The high molecular weight polyarylene polyethers of the present invention are the linear thermoplastic reaction products of an alkali metal double salt of a dihydric phenol and a dihalobenzenoid compound. Characteristically, this polymer has a basic structure composed of recurring units having the formula

-O-E-O-E'-

> wherein E is the residuum of the dihydric phenol and E' is the residuum of the benzenoid compound, both of which are valently bonded to the ether oxygen through aromatic carbon atoms, as hereinafter more fully discussed. Polymers of this type exhibit excellent strength and toughness properties as well as outstanding thermal, oxidative and chemical stability.

The 1963 application then discussed the identity of E and the E precursor compound, that is, the compound which is the predecessor of E in the general formula. It stated:

> The residuum E of the dihydric phenol of these alkali metal salts is not narrowly critical. It can be, for instance, a mononuclear phenylene group as results from hydroquinone and resorcinol, or it may be a di- or polynuclear residuum. Likewise it is possible that the residuum be substituted with other inert nuclear substituents such as halogen, alkyl, alkoxy and like inert substituents.
>
> Such dinuclear phenols can be characterized as having the structure:
> *Graphic material consisting of a chemical formula or diagram set at this point is not available. See text in hard copy or call BNA PLUS at 1-800-452-7773 or 202-452-4323.*
>
> wherein Ar is an aromatic group and preferably is a phenylene group, Y and Y 1can be the same or different inert substituent groups as alkyl groups having from 1 to 4 carbon atoms, halogen atoms, i.e. fluorine, chlorine, bromine or iodine, or alkoxy radicals having from 1 to 4 carbon atoms, r and z are integers having a value from 0 to 4, inclusive, and R is representative of a bond between aromatic carbon atoms as in dihydroxydiphenyl, or is a divalent radical, including for example, inorganic radicals as
> *Graphic material consisting of a chemical formula or diagram set at this point is not available. See text in hard copy or call BNA PLUS at 1-800-452-7773 or 202-452-4323.*
> , -O-, -S-, -S-S-, $-SO_2-$, and divalent organic hydrocarbon radicals such as alkylene, alkylidene, cycloaliphatic, or the halogen, alkyl, aryl or like substituted alkylene, alkylidene and cycloaliphatic radicals as well as alkalicyclic, alkarylene and aromatic radicals and a ring fused to both Ar group[s].

The application then mentioned by name some fifty specific dihydric dinuclear phenol (bisphenol) compounds which could be the E precursor compound. The application further stated:

> A preferred form of the polyarylene polyethers of this invention are those prepared using the dihydric polynuclear phenols of the following four types, including the derivatives thereof which are substituted with inert substituent groups
> *Graphic material consisting of a chemical formula or diagram set at this point is not available. See text in hard copy or call BNA PLUS at 1-800-452-7773 or 202-452-4323.*

in which the R group represents hydrogen, lower alkyl, lower aryl and the halogen substituted groups thereof, which can be the same or different.
Graphic material consisting of a chemical formula or diagram set at this point is not available. See text in hard copy or call BNA PLUS at 1-800-452-7773 or 202-452-4323.

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Turning to the identity of the E' precursor compound, the application stated:

> Any dihalobenzenoid compound or mixture of dihalobenzenoid compounds

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> can be employed in this invention which compound or compounds has the two halogens bonded to benzene rings having an electron withdrawing group in at least one of the positions ortho and para to the halogen group. The dihalobenzenoid compound can be either mononuclear where the halogens are attached to the same benzenoid ring or polynuclear where they are attached to different benzenoid rings, as long as there is the activating electron withdrawing group in the ortho or para position of that benzenoid nucleus.

The 1963 application also included a discussion of the electron withdrawing group that was substantially the same as the paragraphs quoted above from the 1972 application.

The 1963 application contained twenty-six "examples" disclosing in detail the physical and chemical characteristics of fifteen species of polyarylene polyethers. One of the species was the polymer composed of these recurring structural units (which we designate as species [1]): [8]
Graphic material consisting of a chemical formula or diagram set at this point is not available. See text in hard copy or call BNA PLUS at 1-800-452-7773 or 202-452-4323.

Another species disclosed was the polymer composed of these recurring structural units (which we designate as species [2]): [9]
Graphic material consisting of a chemical formula or diagram set at this point is not available. See text in hard copy or call BNA PLUS at 1-800-452-7773 or 202-452-4323.

Appellants' 1963 application became involved in a three-party interference [10] which resulted in an award of priority adverse to appellants from which they did not appeal. [11]"] The sole count of the interference recited species [1].

After their involvement in the interference ended, appellants filed the 1972 application, and they sought broad claims which would at the same time exclude the subject matter of the lost count.

The Claims

Claim 1, now on appeal, is illustrative of the group of claims (claims 1-9, 64, and 68-70) which seek to exclude the subject matter of the lost count and which are involved in the 35 USC 102 or 103 rejection:

> 1. A substantially linear thermoplastic polyarylene polyether composed of recurring units having the general formula:

Graphic material consisting of a chemical formula or diagram set at this point is not available. See text in hard copy or call BNA PLUS at 1-800-452-7773 or 202-452-4323.

where E is the residuum of a dihydric phenol and E' is the residuum of a benzenoid compound having an inert electron withdrawing group in one or more of the positions ortho and para to the valence bonds having a sigma a1value above about +0.7, and where both of said residuum [sic, residua] are valently bonded to the ether oxygens through aromatic carbon atoms *with the provisos that E and E' may not both include a divalent sulfone group and may not both include a divalent carbonyl group linking two aromatic nuclei.* [Emphasis added.]

The first "proviso" in claim 1, that "E and E' may not both include a divalent sulfone group," excludes species [1], the species of the lost count. The second "proviso," that "E and E' * * * may not both include a divalent carbonyl group," excludes species [2], which appellants state is "analogous" or "equivalent" to species [1]. [12]

Claims 64 and 71 are illustrative of the group of claims (claims 64 and 68-72) rejected under 35 USC 112, first and second paragraphs:

64. A substantially linear thermoplastic polyarylene polyether composed of recurring units having the general formula:
Graphic material consisting of a chemical formula or diagram set at this point is not available. See text in hard copy or call BNA PLUS at 1-800-452-7773 or 202-452-4323.

where E is the residuum of a dihydric phenol and E' is the residuum of a

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benzenoid compound having one or more inert electron withdrawing groups in at least one of the position [sic, positions] ortho and para to the valence bonds having a sigma a1value *sufficient to activate a halogen atom* and where both of said residuum [sic, residua] are valently bonded to the ether oxygens through aromatic carbon atoms with the provisos that E and E' may not both include a divalent carbonyl group linking two aromatic nuclei. [Emphasis added.]

71. The process for preparing substantially linear polyarylene polyethers which comprises reacting substantially equimolar amounts of an alkali metal double salt of a dihydric phenol with a dihalobenzenoid compound *having halogen atoms activated by an inert electron withdrawing group* in at least one of the positions ortho and para to the halogen atom, under substantially anhydrous conditions and in the liquid phase of an organic solvent having the formula:
Graphic material consisting of a chemical formula or diagram set at this point is not available. See text in hard copy or call BNA PLUS at 1-800-452-7773 or 202-452-4323.

in which R represents a member of the group consisting of monovalent lower hydrocarbon groups free of aliphatic unsaturation on the alpha carbon atom and, when connected together represents a divalent alkylene group, and Z is an integer from 1 to 2 inclusive. [Emphasis added.]

The Rejections

The sole reference relied upon by the examiner and the board is:

Netherlands 6,408,130 January 18, 1965

Claims 1-9, 64, and 68-70 were rejected under 35 USC 102 or 103 as unpatentable in view of the Netherlands patent, which is a foreign-filed counterpart of appellants' 1963 application.

Before the PTO, appellants conceded that the invention was fully disclosed in the Netherlands patent. However, appellants contended that the claims are entitled to the benefit of the 1963 filing date under 35 USC 120, [13] and therefore the Netherlands patent is not available as a prior art reference.

The examiner and the board were of the view that the claims are not entitled to the 1963 filing date because the presently claimed subject matter is not "described" in the 1963 application as required by the first paragraph of 35 USC 112. [14] As explained by the board:

> The question determinative of the issue at hand is thus whether or not appellants are entitled to the filing date of their parent application Serial No. 295,519, i.e., July 16, 1963. An answer to this question quite obviously depends on what is the invention defined by the instant claims. Is it the same as the one disclosed in [the] parent case or does it differ therefrom in a manner which precludes the instant claims from being afforded the filing date of the parent case?
>
> Under the rationale of the CCPA as set forth in In re Welstead, 59 CCPA 1105, 463 F.2d 1110, 174 USPQ 449 (compare also In re Lukach et al., 58 CCPA 1233, 442 F.2d 967, 169 USPQ 795 , and In re Smith [(I)], 59 CCPA 1025, 458 F.2d 1389, 173 USPQ 679), which we deem controlling, we are constrained to conclude that the present claims are not entitled to the filing date of appellants' parent case Serial No. 295,519. The claims at issue contain provisos that E and E' may not both include a divalent sulfone group and may not both include a divalent carbonyl group linking two aromatic nuclei. The artificial subgenus thus created in the claims is not described in the parent case and would be new matter if introduced into the parent case. It is thus equally "new matter," i.e., matter new to the present application for

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> which no antecedent basis exists in the parent case. Consequently, appellants are not entitled to rely on the filing date of their parent case to support a new subgenus for which no basis exists in the parent case. The reason why appellants now limit their claims to exclude those species eliminated by the provisos, i.e., loss in an interference, is manifestly immaterial.
>
> Having reached the conclusion that appellants are not entitled to the filing date of their parent case for the subject matter defined by the present claims which delineate a new subgenus not described in the parent case, it follows that the Netherlands patent is a valid reference which, by appellants' own admission, fully meets the claims. The indicated rejection of claims 1-9, 64 and 68-70 under 35 U.S.C. 102 as unpatentable over the Netherlands patent is thus affirmed. The alternative reliance by the Examiner on Section 103 is inconsequential, Section 102 of the statute being the epitome of Section 103. In re Pearson, (CCPA), 494 F.2d 1399, 181 USPQ 641 .

Claims 64 and 68-72 were rejected under 35 USC 112, first and second paragraphs. In his Answer, the examiner stated that the claims were rejected under §112, first paragraph, for "being broader than the enabling disclosure" and under §112, second paragraph, [15] for being "broader than the express limitations disclosed as defining the invention." The examiner said the "specific deficiencies of the claims and disclosure" are that the expression "to activate a halogen" (claim 64) is "indefinite" because "it does not specify toward what the activation is" and that "[t]he express disclosure is clearly limited to the sigma[a1] value recited in claim 1, for example: see [[A] and [B]]."

In affirming the examiner on these rejections, the board stated:

> Further, claims 64 and 68-72 stand finally rejected under 35 U.S.C. 112 as being broader than the enabling disclosure (first paragraph) and broader than the express limitations disclosed as

defining the invention (paragraph two).

It is the Examiner's position that "to activate a halogen atom" (claim 64) is indefinite and that the disclosure also is limited to dihalobenzenoid compounds not broadly merely "activated by an inert electron withdrawing group" (claims 68-72) but the activation must have a sigma a2value above about +0.7.

We agree with this rejection. The specification makes it quite clear that a minimum sigma a3activation value of the halogen atoms is required (note especially [[A]]) and an undefined sigma a4value thus lacks the requisite preciseness commensurate with the enablement of the disclosure.

Opinion

I. The Rejections of Claims 64 and 68-72 under §112

Claims 64 and 68-72 were rejected under both the first and second paragraphs of 35 USC 112.

[1]We begin with the rejections under the second paragraph of §112. As stated in In re Moore, 58 CCPA 1042, 1046-1047, 439 F.2d 1232, 1235, 169 USPQ 236, 238 (1971):

> Any analysis in this regard should begin with the determination of whether the claims satisfy the requirements of the second paragraph. * * *
>
> This first inquiry therefore is merely to determine whether the claims do, in fact, set out and circumscribe a particular area with a reasonable degree of precision and particularity. It is here where the definiteness of the language employed must be analyzed -- not in a vacuum, but always in light of the teachings of the prior art and of the particular application disclosure as it would be interpreted by one possessing the ordinary level of skill in the pertinent art. [Footnote omitted.]

The examiner's §112, second paragraph, rejection was premised on the general ground that the claims are "broader than the express limitations disclosed as defining the invention" and on two specific grounds: (a) that the expression "to activate a halogen atom" is "indefinite" because "it does not specify toward what the activation is;" and (b) that "[t]he express disclosure is clearly limited to the sigma[a5] value recited in claim 1, for example: see [[A] and [B]]." The board affirmed and stated: "an undefined sigma a6value thus lacks the requisite *preciseness* * * *." (Emphasis added.)

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Ground (a) focuses on the specific phrase "to activate a halogen atom." But the language is found only in claim 64, not in claims 68-72. Claim 68 recites "a dihalobenzenoid compound having halogen atoms activated by an inert electron withdrawing group," and claims 71 and 72 have a similar recitation. (Claims 69 and 70 depend from claim 68.) Those recitations clearly specify "toward what the activation is," as the examiner would require. Ground (a), therefore, lacks merit with respect to claims 68-72.

[2]Product claim 64 [16]defines the complete polymer structure by describing the constituents partially in terms of their functions in the reaction and by their linkage into the end-product polymer. The specification provides further guidance on the meaning of the E' term:

> It is seen also that as used herein, the E' term defined as being the "residuum of the benzenoid compound" refers to the aromatic or benzenoid residue of the compound *after the removal of the*

halogen atoms on the benzenoid nucleus. [Emphasis added.]

It is also clear from the specification as a whole, that two keys to the polymerization reaction are inert electron withdrawing groups particularly positioned on the benzenoid nucleus and a cumulative sigma a1value attributable to those withdrawing groups which is sufficient to activate a halogen atom on that nucleus. If the sigma a2value is not sufficient to activate a halogen atom on the benzenoid nucleus, the reaction will not take place and the polymer will not be made. See In re Angstadt, 537 F.2d 498, 190 USPQ 214 (CCPA 1976). The specification adequately details which sigma a3values are sufficient to carry out the reaction, and any person skilled in the art would immediately recognize from the above-quoted portion of the disclosure or the specification as a whole that the halogen atom mentioned in claim 64 was on the benzenoid nucleus prior to the reaction. It is clear that those skilled in the art would have no trouble ascertaining whether any particular polymer falls within the scope of claim 64. See In re Goffe, 526 F.2d 1393, 188 USPQ 131 (CCPA 1975). The questioned limitation is merely surplusage, since the claim would be definite with or without it. [17]

[3]The point made by the board, that "an undefined sigma a4value" lacks "preciseness," is also unsound. [18]Claim language must be read in light of the specification as it would be interpreted by one of ordinary skill in the art. In re Moore, supra. As pointed out above, those skilled in the art will be able to determine immediately from appellants' detailed specification what level of activation (i.e., sigma a5value) is necessary to practice the invention. Cf. In re Mattison, 509 F.2d 563, 184 USPQ 484 (CCPA 1975). We conclude that the subject matter embraced by claims 64 and 68-72 is definite and that the claims set out and circumscribe a particular area with a reasonable degree of precision and particularity. In re Angstadt, supra; In re Skoll, 523 F.2d 1392, 187 USPQ 481 (CCPA 1975); In re Watson, 517 F.2d 465, 186 USPQ 11 (CCPA 1975); In re Moore, supra. Therefore, the rejection of claims 64 and 68-72 under the *second* paragraph of 35 USC 112 is reversed.

[4]The examiner's general ground and his ground (b) raise a lack of enablement issue properly arising under the *first,* not the second, paragraph of §112. Ground (b) simply supplies the examiner's reasoning in support of the rejection of the claims under §112, first paragraph, as "broader than the enabling disclosure."

As appellants state, the crux of this lack of enablement rejection is that although the specification describes how the halogen atoms bonded to the dihalobenzenoid compound (the E' precursor compound) must be activated in order for polymerization to occur, the claims at issue do not recite a numerical definition of the degree of activation (a minimum sigma a6value) required from the electron withdrawing group. The PTO position is that the claims must recite a minimum sigma a7value in order to conform the scope of the claims to the scope of enablement provided by the specification. The PTO relies on statements [A] and [B] to prove that the scope of enablement

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provided by the specification is not commensurate with the scope of the claims.

[5]First, we note that it is the function of the specification, not the claims, to set forth the "practical limits of operation" of an invention. In re Rainer, 49 CCPA 1243, 1248, 305 F.2d 505, 509, 134 USPQ 343, 346 (1962). One does not look to claims to find out how to practice the invention they define, but to the specification. In re Roberts, 470 F.2d 1399, 1403, 176 USPQ 313, 315 (CCPA 1973); In re Fuetterer, 50 CCPA 1453, 319 F.2d 259, 138 USPQ 217 (1963).

[6]Second, we note that the specification *as a whole* must be considered in determining whether the scope of enablement provided by the specification is commensurate with the scope of the claims. In re Moore, supra at 1047, 439 F.2d at 1235, 169 USPQ at 238-39.

The present specification includes broad statements such as: "Any electron withdrawing group can be employed as the activator group in these compounds." The specification also discusses preferred embodiments, alternative embodiments, and the practical limits of operation.

Statement [A] describes preferred embodiments and practical limits of operation. It says that electron withdrawing groups having a high sigma a1value ("preferably above 1.0") are preferred and that the practical limit of operation of the polymerization reaction is reached when the electron withdrawing group has a sigma a2value of 0.7 (at that value the reaction rate "may be somewhat low").

Statement [B] describes an alternative embodiment ("a plurality of electron withdrawing groups") and the practical limit of operation for this embodiment. It states that the cumulative sigma a3influence should be "at least about +0.7."

[7]The PTO would limit appellants to claims reciting a sigma a4value of at least 0.7. This view is improper because it requires the claims to set forth the practical limits of operation for the invention and it effectively ignores the scope of enablement provided by the specification as a whole. As we said in In re Goffe, 542 F.2d 564, 567, 191 USPQ 429, 431 (CCPA 1976):

> [T]o provide effective incentives, claims must adequately protect inventors. To demand that the first to disclose shall limit his claims to what he has found will work or to materials which meet the guidelines specified for "preferred" materials in a process such as the one herein involved would not serve the constitutional purpose of promoting progress in the useful arts. See In re Fuetterer, 50 CCPA 1453, 1462, 319 F.2d 259, 265, 138 USPQ 217, 223 (1963). [Footnote omitted.]

The rejection of claims 64 and 68-72 under the *first* paragraph of 35 USC 112 is reversed.

II. The Rejection of Claims 1-9, 64, and 68-70 Under §102 or §103, Raising Issues Under §112 and §120

[8]We are convinced that the invention recited in claim 1 is "disclosed in the manner provided by the first paragraph of section 112" in the 1963 application and that claim 1 is therefore entitled to the benefit of the 1963 filing date. [19]The only inquiry is whether, after exclusion from the original claims of two species specifically disclosed in the 1963 application, the 1963 disclosure satisfies §112, first paragraph, for the "limited genus" [20]now claimed.

While the board found that "no antecedent basis exists in the parent case" for the "limited genus" in claim 1, we see more than ample basis for claims of such scope. The 1963 disclosure is clearly directed to polymers of the type claimed. Fifty specific choices are mentioned for the E precursor compound, a broad *class* is identified as embracing suitable *choices* for the E' precursor compound, and twenty-six "examples" are disclosed which detail fifteen species of polyarylene polyethers. Only fourteen of those species and twenty-three of the "examples" are within the scope of the claims now on appeal. Two of the many choices for E and E' precursor compounds are deleted from the protection sought, because appellant is *claiming less* than the full scope of his disclosure. But, as we said in In re Wertheim, 541 F.2d 257, 263, 191 USPQ 90, 97 (CCPA 1976):

> Inventions are constantly made which turn out not to be patentable, and applicants frequently discover during the course of prosecution that only a part of what they invented and originally claimed is patentable.

[9]It is for the inventor to decide what *bounds* of protection he will seek. In re Saunders, 58 CCPA 1316, 1327, 444 F.2d 599, 607, 170 USPQ 213, 220 (1971). To deny appellants the benefit of their grandparent application in this case would, as this court said in Saunders:

> * * * let form triumph over substance, substantially eliminating the right of an applicant to retreat to an otherwise patentable species merely because he erroneously thought he was first with the genus when he filed.

The board cited as "controlling" the decisions of this court in In re Welstead, 59 CCPA 1105, 463 F.2d 1110, 174 USPQ 449 (1972); In re Lukach, 58 CCPA 1233, 442 F.2d 967, 169 USPQ 795 (1971); and In re Smith, 59 CCPA 1025, 458 F.2d 1389, 173 USPQ 679 (1972). Those decisions, because of important factual distinctions, are not controlling.

In Welstead the applicant was attempting to introduce into his claims a new subgenus where "* * * the specification * * * contained neither a description * * * of the [subgenus] * * * nor descriptions of the species thereof amounting in the aggregate to the same thing * * *." Welstead conceded the absence from his disclosure of compounds of the "second type" within the new subgenus. Welstead is thus clearly distinguishable from the present case, in which appellants' grandparent application contains a broad and complete generic disclosure, coupled with extensive examples fully supportive of the limited genus now claimed. Indeed, Welstead might have well been cited by the board in support of a decision contrary to that reached, in view of what this court there implied concerning the possibility that "descriptions of species amounting in the aggregate to the same thing" may satisfy the description requirements of 35 USC 112, paragraph one.

Similarly, in Lukach we noted that "* * * the grandparent application here does not disclose any defined genus of which the presently claimed copolymers are a subgenus." That is not the fact here. Appellants' grandparent application clearly describes the genus and the two special classes of polymer materials excluded therefrom.

In Smith the applicant sought the benefit of his prior application for a broadened generic claim, replacing the claim limitation "at least 12 carbon atoms * * *" with a new limitation calling specifically for 8 to 36 carbon atoms, where there was no disclosure of either the range itself or of a sufficient number of species to establish entitlement to the claimed range. Appellants, in contrast to the applicant in Smith, are narrowing their claims, and the full scope of the limited genus now claimed is supported in appellants' earlier application, generically and by specific examples.

[10]The notion that one who fully discloses, and teaches those skilled in the art how to make and use, a genus and numerous species therewithin, has somehow failed to disclose, and teach those skilled in the art how to make and use, that genus minus two of those species, and has thus failed to satisfy the requirements of §112, first paragraph, appears to result from a hypertechnical application of legalistic prose relating to that provision of the statute. All that happened here is that appellants narrowed their claims to avoid having them read on a lost interference count.

[11]The board indicated that "it is manifestly immaterial" *why* appellants limited their claims. Though it is true that insufficiency under §112 could not be cured by citing the causes for such insufficiency, it is not true that the factual context out of which the question under §112 arises is immaterial. Quite the contrary. Here, as we hold on the facts of this case, the "written description" in the 1963 specification supported the claims in the absence of the limitation, and that specification, having described the whole, necessarily described the part remaining. The facts of the prosecution are properly presented and relied on, under these circumstances, to indicate that appellants are merely excising the invention of another, to which they are not entitled, and are not creating an "artificial subgenus" or claiming "new matter."

In summary, and for the reasons discussed, the rejections of claims 64 and 68-72 under §112, first and second paragraphs, are reversed; appellants' 1963 disclosure satisfied §112, first paragraph, with respect to claims 1-9, 64, and 68-70 and appellants are, therefore, entitled to the benefit of their 1963 filing date under 35 USC 120. The Netherlands patent is thus rendered unavailable as a prior art reference, and the rejection of the claims under 35 USC 102 or 103 is reversed.

Footnotes

Footnote 1. Claims 10-54 and 65-67 stand allowed. A petition for reconsideration was denied by the board.

Footnote 2. The - O - linkages in the general formula are called ether linkages.

Footnote 3. A dihydric phenol is a type of aromatic organic compound in which two hydroxy (-OH) groups are attached directly to a benzene ring.

Footnote 4. An electron withdrawing group is a substituent which withdraws electrons from the aromatic ring to which it is attached.

Footnote 5. An aromatic ring bearing substituents on adjacent carbon atoms is called ortho substituted.

Footnote 6. An aromatic ring bearing substituents on opposite carbon atoms is called para substituted.

Footnote 7. Appellants' brief specifically refers to one of the publications cited (Chem. Rev., 53, 222 [1953]) and states that its author (Jaffe) defines the sigma a1 value as a "special substituent constant" for the "Hammett equation" which is an empirically derived formula intended to show a general quantitative relation between the nature of a given substituent and the reactivity of a side chain. Thus, sigma a2 values are based on experimental data and they measure the "activation energy" of a given substituent (electron withdrawing group).

Footnote 8. The -SO 2- linking group in species [1] is called a sulfone group.

Footnote 9. The -CO- linking group in species [2] is called a carbonyl group.

Footnote 10. Interference No. 95,807, declared February 17, 1967.

Footnote 11. Another party did appeal. See Vogel v. Jones, 486 F.2d 1068, 179 USPQ 425 (CCPA 1973).

Footnote 12. The provisos actually exclude more than species [1] and [2]. For example, polymers similar to species [1] and [2] but having substituted ring structures are also excluded.

Footnote 13. §120. Benefit of earlier filing date in the United States.

An application for patent *for an invention disclosed in the manner provided by the first paragraph of section 112 of this title in an application previously filed in the United States* by the same inventor shall have the same effect, *as to such invention,* as though filed on the date of the prior application, if filed before the patenting or abandonment of or termination of proceedings on the first application or on an application similarly entitled to the benefit of the filing date of the first application and if it contains or is amended to contain a specific reference to the earlier filed application. [Emphasis added.]

Footnote 14.

§112. Specification.

The specification shall contain *a written description of the invention,* and of the manner and process of making and using it, *in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same,* and shall set forth the best mode contemplated by the inventor of carrying out his invention. [Emphasis added.]

Footnote 15.

§112. Specification.

* * *

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Footnote 16.

Claims 68-70 are product-by-process claims.

Footnote 17. We do not speculate on whether or not the claim would be unduly broad if the questioned limitation were removed. But undue breadth is not indefiniteness. In re Borkowski, 57 CCPA 946, 422 F.2d 904, 164 USPQ 642 (1970). This claim is definite either with or without the phrase "to activate a halogen atom."

Footnote 18. In re Merat, 519 F.2d 1390, 186 USPQ 471 (CCPA 1975), cited by the Solicitor, affirmed a §112, second paragraph, rejection because the same word ("normal") was used in the claims in one sense and in the specification in a different sense, thus rendering the claims indefinite. There is nothing akin to the Merat situation here.

Footnote 19. Appellants have not argued the claims separately, thus, claims 2-9, 64, and 68-70 stand or fall with claim 1.

Footnote 20. Appellants refer to the subject matter recited in claim 1 as a "limited genus." The board called it an "artificial subgenus." We use appellants' terminology. Whatever the label, the issue is the same.

Dissenting Opinion Text

Dissent By:
Lane, Judge, dissenting in part,

I would affirm the rejection of claims 64 and 68-72 under §112, paragraphs 1 and 2, because the specification indicates that a

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minimum sigma value of +0.7 is an *essential requisite.* These claims fail to recite this requisite, thus fail to define appellants' invention and are broader than the disclosure. I concur in reversing the rejection of claims 1-9.

- End of Case -

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Purdue Pharma L.P. v. Faulding Inc., 56 USPQ2d 1481 (CA FC 2000)

56 USPQ2D 1481
Purdue Pharma L.P. v. Faulding Inc.

U.S. Court of Appeals Federal Circuit

Nos. 99-1416,-1433
Decided October 25, 2000

Headnotes

PATENTS

[1] Patentability/Validity — Obviousness — References and claims as whole (§115.0904)

Patentability/Validity — Specification — Written description (§115.1103)

Limitation in patent for sustained release, oral morphine drug formulation, which requires "a maximum plasma concentration . . . which is more than twice the plasma level of said opioid at about 24 hours administration of the dosage form," was not adequately described in disclosure of application as originally filed, since language describing invention as not having "generally flat" or "substantially flat"morphine plasma concentration curve refers to rapid opioid release feature recited in original claims, not to limitation in question, and person of ordinary skill in art would not understand that language to denote concentration profile required by limitation in any event, and since examples set forth in patent do not emphasize ratio claimed in limitation, or direct one of ordinary skill in art to that ratio as important aspect of invention; general disclosure of genus of compounds does not support claim directed to single compound, and patentees cannot pick characteristic possessed by two of their formulations and then make it basis of claims that cover any formulation having that characteristic.

[2] Patentability/Validity — Specification — Written description (§115.1103)

Federal district court did not commit errors of law in finding that claims of patent for sustained release, oral morphine drug formulation are unsupported by adequate written description, since court noted that it was compelled to consider all examples in patent collectively, given that specification did not state whether any particular examples pertained to invention of asserted claims, but court did not improperly

insist that examples identify exactly what constitutes invention and what does not, since court viewed disclosure as whole in concluding that specification did not support asserted claims, and since court did not improperly look to written description, rather than amended claims, to define invention.

[3] Patentability/Validity — Specification — Written description (§115.1103)

JUDICIAL PRACTICE AND PROCEDURE

Procedure — Judicial review — Standard of review —Patents (§410.4607.09)

Federal district court, in finding that asserted claims are unsupported by adequate written description, did not err by failing to defer to patent examiner's statement that claims at issue "are supported by the specs," since court understood that question of compliance with written description requirement is one of fact, and since court did not find examiner's statement persuasive in light of all evidence in case; district court was not required to sustain examiner's decision as long as it was supported by substantial evidence, since that standard of review has no application in infringement action that originated in district court, although patentability decision of U.S. Patent and Trademark Office is accorded deference in district court litigation.

Particular patents — Chemical — Pain relief

5,672,360, Sackler, Kaiko, and Goldenheim, method for treating pain by administering 24 hour oral opioid formulations, judgment of invalidity affirmed.

Case History and Disposition
Appeal from the U.S. District Court for the District of Delaware, Farnan, C.J.

Action by Purdue Pharma L.P. and Purdue Frederick Co. against Faulding Inc., Faulding Pharmaceutical Co., Faulding Services Inc., and Purepac Pharmaceutical Co. for patent infringement. Following bench trial, federal district court found that defendants had infringed asserted claims but that claims were invalid, and parties cross-appealed. Judgment affirmed as to finding of invalidity.

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Pennie & Edmonds, Washington, D.C., for plaintiffs-appellants.

Steven J. Lee, Paul H. Heller, Edward J. Handler III, Charles A. Weiss, William G. James II, and Mark I. Koffsky, of Kenyon &Kenyon, New York; E. Brendan Magrab, of Faulding Inc., Elizabeth, N.J.; Jack B. Blumenfeld and Karen Jacobs Louden, of Morris, Nichols, Arsht & Tunnell, Wilmington, Del., for defendants-cross appellants.

Judge:
Before Plager, circuit judge, Smith, senior circuit judge, and Bryson, circuit judge.

Opinion Text

Opinion By:
Bryson, J.

Purdue Pharma L.P. and The Purdue Frederick Company (collectively Purdue) own U.S. Patent No. 5,672,360 (the '360 patent), which is drawn to methods of treating pain in patients by administering an opioid, such as morphine, once a day. Purdue brought a patent infringement suit against Faulding Inc., Faulding Pharmaceutical Co., Faulding Services, Inc., and Purepac Pharmaceutical Co. (collectively Faulding) in the United States District Court for the District of Delaware. After a bench trial, the district court found that Faulding had infringed the asserted claims of the '360 patent but that the claims were invalid. Purdue appeals from the finding of invalidity, and Faulding cross-appeals from the finding of infringement. We uphold the court's ruling invalidating the asserted claims of the '360 patent; we do not reach Faulding's cross-appeal on the issue of infringement.

I

In 1984 Purdue introduced a sustained-release, twice-a-day oral morphine formulation. Sustained-release formulations represent a significant advance over immediate-release morphine formulations because immediate-release formulations need to be administered every four hours, a schedule that interferes with the patient's sleep and subjects the patient to cycles of pain that are difficult to control.

After its success with its twice-a-day formulation, Purdue sought to develop a sustained-release oral morphine formulation that would need to be administered only once a day. The work of its researchers initially led to the issuance of U.S. Patent No. 5,478,577 (the '577 patent), which discloses a once-a-day formulation exhibiting a rapid initial rise in the opioid concentration in the patient's blood.

During the same period, Faulding was developing long-lasting opioid anti-pain formulations as well. In 1996, Faulding began marketing its oral sustained-release morphine formulation in the United States under the trade name Kadian. The package insert accompanying Kadian states that it may be administered either once or twice a day.

Shortly after Faulding began selling Kadian in this country, Purdue brought suit against Faulding and Zeneca Inc., alleging that the manufacture, sale, and use of Kadian as a once-a-day morphine formulation infringed the '577 patent. At the time the suit was filed, the inventors of the '577 patent had pending before the Patent and Trademark Office U.S. Patent Application Serial No. 08/578,688 (the '688 application), which claimed priority to the application that led to the '577 patent.

While the litigation over the '577 patent was pending, Purdue's counsel canceled the pending claims of the '688 application and amended the application to add all new claims. The application was allowed as amended, and it issued as the '360 patent on September 30, 1997. No art rejections were made against the issued claims. The only prosecution history is contained in a handwritten interview summary in which the examiner stated that the "new claims are supported by the specs."

Purdue asserts that the once-a-day formulation described in the treatment method of the '360 patent, which results in a substantial fluctuation in the opioid concentration in the patient's blood between the maximum concentration level and the concentration level at the end of the 24-hour dosage period, was contrary to the prevailing view at the time that sustained-release formulations should produce minimal fluctuations in the opioid concentration level during the dosing interval. That aspect of the invention is reflected in each of the claims of the '360 patent, including claims 2, 4, and 11, the three asserted claims at issue in this case. Claims 1 and 9, on which the three asserted claims depend, both contain a limitation requiring that the maximum plasma concentration of the opioid be more than twice the plasma level of the opioid 24 hours after administration of the drug. The pertinent claims of the '360 patent at issue in this case read as follows:

1. A method of effectively treating pain in humans, comprising orally administering

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to a human patient on a once-a-day basis an oral sustained release dosage form containing an opioid analgesic or salt thereof which upon administration provides a time to maximum plasma concentration (T_{max}) of said opioid in about 2 to about 10 hours and a maximum plasma concentration (C_{max}) which is more than twice the plasma level of said opioid at about 24 hours after administration of the dosage form, and which dosage form provides effective treatment of pain for about 24 hours or more after administration to the patient.
2. The method of claim 1, wherein the T_{max} occurs in about 2 to about 8 hours after oral administration of said dosage form.
4. The method of claim 1, wherein said opioid analgesic is morphine sulfate.
I. A method of effectively treating pain in humans, comprising orally administering to a human patient on a once-a-day basis an oral sustained release dosage form containing an opioid analgesic or salt thereof which at steady-state provides a time to maximum plasma concentration (T_{max}) of said opioid in about 2 to about 10 hours and a maximum plasma concentration (C_{max}) which is more than twice the plasma level of said opioid at about 24 hours after administration of the dosage form, and which dosage form provides effective treatment of pain for about 24 hours or more after administration to the patient.
11. The method of claim 9, wherein said opioid analgesic is morphine sulfate.
Shortly after the '360 patent issued, Purdue amended the complaint in the pending litigation against Faulding and Zeneca by dropping its claims under the '577 patent and asserting infringement of the '360 patent. Faulding and Zeneca asserted various counterclaims, including non-infringement and invalidity, and a bench trial was held on liability. During trial, the district court dismissed the claims against Zeneca. Following the trial, the court held that Faulding's production and sale of Kadian infringed the asserted claims of the '360 patent, but that the claims were invalid because they lacked the written description required by 35 U.S.C. § 112, first paragraph. The court then entered final judgment on the tried issues under Fed. R. Civ. P. 54(b).

II

The validity issue in this case is whether the limitation "a maximum plasma concentration (C_{max}) which is more than twice the plasma level of said opioid at about 24 hours after administration of the dosage form [C_{24}]" was adequately described in the disclosure of the '688 application as originally filed. The trial court found that it was not.

In order to satisfy the written description requirement, the disclosure as originally filed does not have to provide *in haec verba* support for the claimed subject matter at issue. *See Fujikawa v. Wattanasin*, 93 F.3d 1559, 1570, 39 USPQ2d 1895, 1904(Fed. Cir. 1996). Nonetheless, the disclosure "must ... convey with reasonable clarity to those skilled in the art that ... [the inventor] was in possession of the invention." *Vas-Cath Inc. v. Mahurkar*, 935 F.2d 1555, 1563-64, 19 USPQ2d 1111, 1117(Fed. Cir. 1991). Put another way, one skilled in the art, reading the original disclosure, must "immediately discern the limitation at issue"in the claims. *Waldemar Link GmbH & Co. v. Osteonics Corp.*, 32 F.3d 556, 558, 31 UPSQ2d 1855, 1857 (Fed. Cir. 1994). That inquiry is a factual one and must be assessed on a case-by-case basis. *See Vas-Cath*, 935 F.2d at 1561, 19 USPQ2d at 1116 ("Precisely how close the original description must come to comply with the description requirement of § 112 must be determined on a case-by-case basis."). When the question whether a patent satisfies the written description requirement is resolved by a district court sitting as the trier of fact, we review the court's decision for clear error. *See Tronzo v. Biomet, Inc.*, 156 F.3d 1154, 1158, 47 USPQ2d 1829, 1832(Fed. Cir. 1998); *Gentry Gallery, Inc. v. Berkline Corp.*, 134 F.3d 1473,

1479, 45 USPQ2d 1498, 1502(Fed. Cir. 1998).

Purdue contends that the district court made various legal errors in its analysis of the written description issue and that its factual finding on that issue was clearly erroneous. Turning first to the district court's factual analysis, we conclude that the court's finding on the written description issue did not constitute clear error.

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A

The district court found that the specification of the '360 patent fails to convey that the C_{max}/C_{24} limitation was encompassed within Purdue's original invention. Purdue attacks that finding on several fronts, but its arguments are unpersuasive.

1

[1] Purdue first argues that the C_{max}/C_{24} limitation is supported by the portion of the specification that describes the invention as not having a "generally flat"or "substantially flat" morphine plasma concentration curve. The passage of the specification on which Purdue relies reads as follows:

The state-of-the-art approach to controlled release opioid therapy is to provide formulations which exhibit zero order pharmacokinetics and have minimal peak to trough fluctuation in opioid levels with repeated dosing. This zero order release provides very slow opioid absorption, and a generally flat serum concentration curve over time. A flat serum concentration curve is generally considered to be advantageous because it would in effect mimic a steady-state level where efficacy is provided but side effects common to opioid analgesics are minimized. ...

It has now been surprisingly discovered that quicker and greater analgesic efficacy is achieved by 24 hour oral opioid formulations which do not exhibit a substantially flat serum Concentration curve, but which instead provide a more rapid initial opioid release so that the minimum effective analgesic concentration can be more quickly approached in many patients who have measurable if not significant pain at the time of dosing. ... Also surprising and unexpected is the fact that while the methods of the present invention achieve quicker and greater analgesic efficacy, there is not a significantly greater incidence in side effects which would normally be expected as higher peak plasma concentrations occur. '360 patent, col. 5, ll. 24-55. The district court disagreed with Purdue's argument that the phrase "formulations which do not exhibit a substantially flat serum Concentration curve" refers to the C_{max}/C_{24} ratio of more than two that was added in the amended claims. Instead, the court concluded that the term refers to the feature of rapid opioid release that was recited in the original claims of the application and was described in the specification as "critical" to the invention. The court's finding is supported by the context in which the statement appears, and it is consistent with the claims as originally filed, which defined the formulation as providing "an initially rapid rise ... by providing an absorption half-life [*i.e.*, the time required for one-half of the absorbable opioid to be absorbed into the plasma] from about 1 to 8 hours."

In addition to finding that the "substantially flat" language in the specification did not refer to the C_{max}/C_{24} limitation, the trial court found that even if that language were understood to relate to the fluctuation in opioid concentration in the blood between the maximum concentration level and the concentration level after 24 hours, one skilled in the art would not understand the term "substantially flat"to mean a fluctuation of 100% or less.

At trial, Purdue offered expert testimony that the term "flat" is understood in the field to mean a fluctuation of 100% or less in the concentration of opioid between the maximum level and the level after 24 hours, *i.e.*, a C_{max}/C_{24} ratio of two or less. The court, however, was unpersuaded. As the court explained, one of Purdue's experts, Dr. Goldenheim, described another sustained-release morphine formulation, Roxanol SR, as having a "flat" serum concentration curve, even though he acknowledged that it has a fluctuation of over 100%. In addition, the court found that the publications relied upon by Purdue did not substantiate Purdue's assertion that "flat" means fluctuations of 100% or less. Moreover, the court stated that even if it accepted Purdue's argument that "flat" means a fluctuation of 100% or less, "the use of the modifier `substantially' in the specification, indicates that the word `flat' as used in the '360 patent specification, does not even refer to the precise quantification urged by Purdue."

One of the publications Purdue relied on at trial was International Publication Number WO 94/22431, on which Kabi Pharmacia AB was the applicant. The Kabi application provides pharmacokinetic profiles for two different morphine formulations, CR-A and CR-B. The trial court found that for the CR-A formulation the C_{max} level was more than twice as great as the C_{24}level, and that for the CR-B

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formulation the C_{max} level was less than twice as great as the C_{24} level. Nonetheless, the Kabi application described both formulations as having "low" fluctuations. The court therefore found that the Kabi application "fails to support Purdue's contention that one skilled in the art understands `flat' to mean fluctuations of less than 100%."

Purdue argues that Kabi's CR-A is a twice-a-day formulation and that the court's reliance on that formulation was therefore misplaced. As noted by Faulding, however, the data in the Kabi application was based on the administration of a single dose of morphine. For that reason, the court was not mistaken in relying on the description of the C_{max}/C_{24} ratio for the CR-A formulation in concluding that the Kabi application fails to support Purdue's argument that one skilled in the art would interpret "substantially flat" to mean a C_{max}/C_{24} ratio of two or less.

Purdue also argues that the trial court was confused with respect to Dr. Goldenheim's testimony regarding Roxanol SR, which Dr. Goldenheim characterized as having a flat profile. Purdue argues that Roxanol SR is approved only as an eight-hour formulation and that the C_{max}/C_8ratio of Roxanol SR is less than 2. On cross-examination, however, Dr. Goldenheim was asked to calculate a C_{max}/C_{12}ratio for Roxanol SR from an article containing pharmacokinetic studies of the drug. From the data presented in the paper, Dr. Goldenheim determined that the C_{max}/C_{12} ratio for Roxanol SR is greater than two, and he characterized that C_{max}/C_{12} ratio as "pretty flat."

That evidence is meaningless, Purdue asserts, because Roxanol is not described as being approved for twice-a-day administration. Dr. Goldenheim's testimony on cross-examination, however, related to the morphine concentration in the Roxanol SR formulation after 12 hours, and the district court reasonably interpreted Dr. Goldenheim's testimony as a concession that a C_{max}/C_{12} ratio greater than two would still be considered "flat."From that evidence, the district court permissibly concluded that a person skilled in the art would not necessarily interpret the term "flat"to be limited to a concentration level ratio less than or equal to two.

Finally, Purdue asserts that the trial court erroneously failed to consider the teachings of the Morella patents. Those patents, Purdue contends, establish that by 1993 it was understood in the field that a flat

pharmacokinetic profile constituted a profile having fluctuations of 100% or less. For example, Purdue argues, U.S. Patent No. 5,202,128, to Morella et al. states that an advantage of the morphine formulations of the invention is that the peak-to-trough variation will be between 60% and 100%, which has been described as a "flat plasma morphine concentration time profile." Purdue, however, does not point to anything in the Morella patents that suggests that if the peak-to-trough variation is greater than 100%, the concentration profile would not be considered flat. The Morella patents therefore do not in any way undermine the district court's finding that a person of ordinary skill in the art would not understand the term "substantially flat" to denote a C_{max}/C_{24} ratio of two or less.

2

Purdue argues that even if the passage from the specification referring to the "substantially flat serum Concentration curve" does not provide the required written description for the C_{max}/C_{24} ratio recited in the claims, the examples set forth in the patent provide adequate support for that limitation. Purdue relies on Example 1 (fed and fasted) and Example 3 (fed only) to support the claimed limitation, as the morphine formulation in both examples resulted in a C_{max}/C_{24}ratio greater than two.

The district court rejected Purdue's argument, pointing out that the specification also contains examples in which the C_{max}/C_{24} ratio is less than two and that nothing in the specification indicates to the skilled artisan which examples embody the claimed invention and which do not. We conclude that the district court did not commit clear error in finding that the examples do not provide sufficient support for the C_{max}/C_{24} limitation.

The specification sets forth seven examples. Values for $C_{max}$and C_{24} are provided for only the first three. Other pharmacokinetic data are provided as well, and morphine concentrations are provided for times other than 24 hours after administration of the drug. Although the examples provide the data from which one can piece together the C_{max}/C_{24} limitation, neither the text accompanying the examples, nor the data, nor anything else in the specification in any way emphasizes the C_{max}/C_{24} ratio. The district court therefore reasonably concluded that one of ordinary

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skill in the art would not be directed to the C_{max}/C_{24} ratio as an aspect of the invention.

The case of *In re Ruschig*, 379 F.2d 990, 154 USPQ 118 (CCPA 1967), is instructive here. In that case our predecessor court affirmed the holding of the Patent Office Board of Appeals that one of the claims, adopted for purposes of interference, was not supported by the disclosure. The claim at issue in that case was directed to a single compound. The applicants argued that, although the compound itself was not disclosed, one skilled in the art would find support for the claimed compound in the general disclosure of the genus of compounds to which the claimed compound belonged. The *Ruschig* court rejected that argument, stating that

[i]t is an old custom in the woods to mark trails by making blaze marks on the trees. It is of no help in finding a trail or in finding one's way through the woods where the trails have disappeared—or have not yet been made, which is more like the case here—to be confronted simply by a large number of unmarked trees. We are looking for blaze marks which single out particular trees. We see none. *Id.* at 994-95, 154 USPQ at 122. Although this case differs from *Ruschig* in that what was disclosed in *Ruschig* was a genus encompassing potentially half a million compounds, the rationale applies equally to this case, in which the disclosure of the '360 patent discloses a multitude of pharmacokinetic parameters,

with no "blaze marks" directing the skilled artisan to the C_{max}/C_{24} ratio or what value that ratio should exceed. *See id.* at 994, 154 USPQ at 122 ("Specific claims to single compounds require reasonably specific supporting disclosure and while we agree with the appellants, as the board did, that naming is not essential, something more than the disclosure of a class of 1000, or 100, or even 48, compounds is required."). As *Ruschig*makes clear, one cannot disclose a forest in the original application, and then later pick a tree out of the forest and say "here is my invention."In order to satisfy the written description requirement, the blaze marks directing the skilled artisan to that tree must be in the originally filed disclosure. *See id.* at 994-95, 154 USPQ at 122; *Fujikawa,* 93 F.3d at 1570-71, 39 USPQ2d at 1905; *Martin v. Mayer*, 823 F.2d 500, 505, 3 USPQ2d 1333, 1337(Fed. Cir. 1987) ("It is 'not a question of whether one skilled in the art might be able to construct the patentee's device from the teachings of the disclosure. ... Rather, it is a question whether the application necessarily discloses that particular device.'") (quoting *Jepson v. Coleman*, 314 F.2d 533, 536, 136 USPQ 647, 649-50(CCPA 1963)). Under that standard, we conclude that the district court did not commit clear error in finding that nothing in the '688 application "'necessarily' ...described the later claimed subject matter" of the '360 patent. *In re Daniels*, 144 F.3d 1452, 1456, 46 USPQ2d 1788, 1790(Fed. Cir. 1998).

In the case of the '360 patent, there is nothing in the written description of Examples 1 and 3 that would suggest to one skilled in the art that the C_{max}/C_{24} ratio is an important defining quality of the formulation, nor does the disclosure even motivate one to calculate the ratio. For example, the description of Example 1 states that

[p]lasma morphine concentrations were used for calculation of phamacokinetic parameters including: (a) absorption and elimination rates; (b) area under the curve (AUC); (c) maximum plasma concentration (C_{max}); (d) time to maximum plasma concentration [()T_{max}); (e) $T_{1/2}$ (elimination). '360 patent, col. 16, ll. 24-29. Figure 9 of the patent graphically represents the mean morphine plasma concentration-time profile for Examples 1 and 2, as well as for the control formulation, MS-Contin. In discussing Figure 9, the disclosure merely states that "it can be seen that the formulation of Example 1 attains a higher and earlier C_{max} but a slightly lower extent of morphine absorption than the formulation of Example 2." *Id.* at col. 21, ll. 8-11.

These statements and the calculation of the listed pharmacokinetic parameters are consistent with how the inventors characterize the invention, as the specification states earlier that "inventive sustained release once-a-day formulations may be characterized by the fact that they are designed to provide an initially rapid rate of rise in the plasma concentration of said opioid characterized by providing an absorption half-life from about 1 to about 8 hours," '360 patent, col. 6, ll. 1-5, and also that "the inventive formulations may be further characterized by having a surprisingly fast time to peak drug concentration (*i.e.*, t_{max})," *id.* at col. 6, ll. 10-12. As can be seen from these excerpts from the specification, however, there is nothing in the written disclosure

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as originally filed directing the skilled artisan to the C_{max}/C_{24} ratio.

What the '360 patentees have done is to pick a characteristic possessed by two of their formulations, a characteristic that is not discussed even in passing in the disclosure, and then make it the basis of claims that cover not just those two formulations, but any formulation that has that characteristic. This is exactly the type of overreaching the written description requirement was designed to guard against. *See Vas-Cath*, 935 F.2d at 1561, 19 USPQ2d at 1115 ("Adequate description of the invention guards against the inventor's overreaching by insisting that he recount his invention in such detail that his future claims can be determined to be encompassed within his original creation.") (quoting *Rengo Co. v. Molins Mach. Co.*, 657 F.2d 535, 551, 211 USPQ 303, 321(3d Cir. 1981)).

3

Purdue characterizes this case as one in which, at bottom, the applicants claimed less than they disclosed. Using the data from Examples 1 and 3, the skilled artisan can establish a range for the C_{max}/C_{24} ratio of 1.28 to 3.43. Thus, according to Purdue, the claim limitation requiring C_{max}/C_{24} to be greater than two is narrower than the range disclosed in the specification. Purdue asserts that it did not consider claims in which the C_{max}/C_{24} ratio was less than two to be patentable in light of the prior art, and that its willingness to settle for claims narrower than the invention it disclosed does not create a written description problem.

Because the specification does not clearly disclose to the skilled artisan that the inventors of the '360 patent considered the C_{max}/C_{24} ratio to be part of their invention, it is immaterial what range for the C_{max}/C_{24} ratio can be gleaned from the examples when read in light of the claims. There is therefore no force to Purdue's argument that the written description requirement was satisfied because the disclosure revealed a broad invention from which the claims carved out a patentable portion.

B

Apart from the asserted factual flaws in the district court's analysis, Purdue contends that the trial court committed several errors of law that affected the court's analysis of the written description issue and require reversal. We have examined each of the claimed legal errors and conclude that the district court did not commit any error of law that had a material effect on the court's judgment.

1

[2] First, Purdue argues that the district court applied the wrong legal test for determining whether the written description requirement was satisfied. Purdue acknowledges that the district court recited the correct test, as set forth in this court's decision in the *Vas-Cath* case, *supra*, but argues that the court actually applied a different test—one that was specifically rejected in *Vas-Cath*. In particular, Purdue relies on a statement in the district court's opinion in which the court commented that "viewing the examples collectively, as the Court believes must be done because there is no way to determine which embody the invention and which do not, the examples illustrate a range between 1.48 and 3.43." That comment, according to Purdue, shows that the district court required the specification to set forth what the invention is and what it is not, which is not the correct test under the written description requirement.

Purdue has misinterpreted the quoted passage from the district court's opinion. The court did not insist that the examples identify exactly what constitutes the claimed invention and what does not; instead, the court simply noted that it had to view all of the examples collectively because the specification did not state that any particular examples pertained to the invention that was recited in the amended claims. Under the circumstances, it was entirely appropriate for the district court to view all of the examples together in its effort to determine whether the disclosure as filed contained a sufficient written description of the invention; indeed, that approach was necessary in order for the court to determine that the inventor "had possession at that time of the later claimed subject matter." *Vas-Cath*, 935 F.2d at 1563, 19 USPQ2d at 1116.

Purdue makes the related contention that the district court did not view the disclosure as a whole in determining whether the written description requirement was satisfied. Again, we read the district court's opinion differently. Although the district court discussed the examples and the text of the specification separately, it is clear from the court's opinion that it concluded that the specification as a whole did not

support the asserted claims of the '360

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patent; there is nothing in the court's opinion suggesting that the court considered that any one segment of the specification, standing alone, had to provide the full support for the amended claims.

2

Purdue next argues that the district court committed legal error by looking to the written description portion of the patent, rather than the claims, to define the invention for purposes of the written description analysis. The district court made no error in this regard. The court noted that it "must necessarily look to the claim language to determine if the specification supports what is now claimed," and it further explained that it could not consider the amended claims themselves, which did not appear in the application as filed, "to show that at the time of filing the inventor was in possession of what is now claimed." We interpret those remarks as simply articulating the correct legal principles that the amended claims define the invention, that the support for the invention must be found in the specification as filed, and that the amended claims could not be used to provide that support.

3

Finally, Purdue contends that the district court improperly disregarded the findings of the examiner, who stated in an interview summary at the time the amended claims were added to the application that the new claims "are supported by the specs." Purdue argues that the district court should have deferred to the examiner's finding on that issue and that the district court failed to do so because the court improperly regarded the written description issue to be an issue of law rather than an issue of fact.

[3] It is true that the district court at one point in its opinion characterized validity as an issue of law. Notwithstanding that isolated statement, the court's lengthy and thorough opinion makes it abundantly clear that the court understood that the question whether the written description requirement was satisfied is a question of fact. Moreover, the district court expressly addressed the examiner's statement on which Purdue relies and found it insufficient on the merits to carry the day for Purdue. The court explained that it did not regard the examiner's cryptic statement as directly applicable to the written description requirement but added that even if the examiner's statement was directed to the written description requirement, "any deference due to the Patent Examiner has been overcome by Faulding's clear and convincing evidence that the specification does not support the asserted claims of the '360 Patent." Thus, the court rejected the examiner's statement on which Purdue relies not because of a misconception about the nature of the issue before it, but because the court did not find the examiner's statement persuasive in light of all the evidence in the case.

Relying on the Supreme Court's decision in *Dickinson v. Zurko*, 527 U.S. 150, 50 USPQ2d 1930 (1999), Purdue makes the related argument that the district court should have sustained the examiner's decision on the written description issue as long as it was supported by substantial evidence. The short answer to that argument is that this was an infringement action that originated in the district court, not an appeal from a decision of the Patent and Trademark Office Board of Appeals and Interferences, which was at issue in *Zurko*. The Administrative Procedure Act standard of review adopted in *Zurko* therefore has no application here. To be sure, as we have noted, the decision of the Patent and Trademark Office with respect to patentability is accorded deference in district court litigation, deference that takes the form of the presumption of validity that is accorded to issued patents under 35 U.S.C. § 282. *See Fromson v. Advance Offset Plate, Inc.*, 755 F.2d 1549, 1555, 225 USPQ 26, 31 (Fed. Cir. 1985). The court, however, was not bound by the examiner's finding in the *ex parte* application proceeding that the new claims were supported by the specification, particularly in light of the fact that the court heard extensive evidence on

the issue in an adversary hearing, none of which was before the patent examiner.

III

Because we have upheld the district court's determination that the asserted claims of the '360 patent are invalid, it is unnecessary to address Faulding's cross-appeal from the district court's finding of infringement.

AFFIRMED.

- End of Case -

ISSN 1526-8535

Ex parte Grasselli, et al., 231 USPQ 393 (BdPatApp&Int 1983)

Ex parte Grasselli, et al.

(BdPatApp&Int)
231 USPQ 393

Decided June 30, 1983
Released May 16, 1986
U.S. Patent and Trademark Office, Board of Patent Appeals and Interferences

Headnotes

PATENTS

1. Patentability -- Invention -- Specific cases -- Chemical (§ 51.5093)

Utility (§ 51.75)

Rejection for obviousness, of claims in application for catalyst, is reversed, in view of evidence demonstrating that one reference relied upon requires sulfur and that remaining references relied upon do not strongly motivate one skilled in art to eliminate such sulfur-containing compound or to expect advantageous result from doing so, but rejection is affirmed on basis of lack of enablement and lack of description, since negative limitations recited in claims, which did not appear in specification as filed, introduce new concepts and violate description requirement of 35 USC 112.

Particular patents -- Catalysts

Grasselli, Suresh, and Miller, application, Ammoxidation of Propane of Isobutane, rejection of claims 1-6 affirmed.

Case History and Disposition:

Appeal from Art Unit 121.

Application for patent of Robert K. Grasselli, Dev D. Suresh, and Robert C. Miller, Serial No. 260,140, filed May 4, 1981, Continuation of Serial No. 148,185, filed May 7, 1980, Continuation of Serial No. 783,999, filed April 4, 1977, Continuation of Serial No. 364,250, filed May 20, 1973. From decision rejecting claims 1-6, applicant appeals. Affirmed.

[Ed. Note: This decision was affirmed by the U.S. Court of Appeals for the Federal Circuit in an unpublished memorandum opinion, 738 F.2d (1984)].

Attorneys:
J.E. Miller, Jr., et al., for appellants.

Joseph P. Brust, Primary Examiner, for Patent and Trademark Office.

Judge:
Before Blech, Goldstein, and Seidleck, Examiners-in-Chief.

Opinion Text

Opinion By:
Goldstein, Examiner-in-Chief.

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This appeal is from the examiner's final rejection of claims 1 through 6. There are no allowed claims. Illustrative claim 1 is reproduced below.

1. In the process for the ammoxidation of propane or isobutane to obtain acyrlonitrile or methacrylonitrile by contacting a mixture of propane or isobutane, ammonia and molecular oxygen at a temperature of about 350°C, to about 650°C with an oxidation catalyst in the absence of sulfur and halogen, the improvement comprising using as the oxidation catalyst a catalyst wherein the atomic ratios of the elements are described by the formula
Graphic material consisting of a chemical formula or diagram set at this point is not available. See text in hard copy or call BNA PLUS at 1-800-452-7773 or 202-452-4323.

wherein A is phosphorous, boron, Ni, Co, alkali metal, alkaline earth metal or mixture thereof;

B is iron, vanadium, manganese, chromium or mixture thereof;

C is molybdenum, tungsten or mixture thereof; and

wherein a is 0 to about 3;

b is 0.01 about 10;

c is 0.1 to about 20;

d is 0.1 to about 10; and

x is the number of oxygens required to satisfy the valence requirements of the other elements present,

said catalyst being free of uranium and the combination of vanadium and phosphorus.

References relied on by the examiner on appeal are:

Table set at this point is not available. See table in hard copy or call BNA PLUS at 1-800-452-7773 or 202-452-4323.

Claims 1 through 6 have been finally rejected under 35 U.S.C. 103 as being obvious from the combined teachings of all the references cited above. We shall not affirm this rejection.

The reference primarily relied upon by the examiner and that which discloses the most closely related catalyst to the one recited in the present claims is Taylor '267. This reference also requires the presence of sulfur or a sulfur-containing compound. Although the remaining references deal with somewhat similar catalysts and generally do not require the presence of a sulfur-containing compound, they would not strongly motivate one of ordinary skill in the relevant art to eliminate the sulfur from the Taylor '267 catalyst system. To whatever extent they might do so, they would certainly not lead one of ordinary skill in the relevant art to expect an advantageous result to occur. Albeit we agree with the examiner that appellants' showing in this regard is not very broad, when combined with the lack of motivation in the first instance to eliminate sulfur from the Taylor '267 catalyst, we consider it adequate to negate the possible existence of a prima facie case of obviousness within the meaning of 35 U.S.C. 103.

We also note that many of the remaining references required the presence of other elements expressly excluded from the present claims, i.e., halogen, uranium or the co-presence of vanadium and phosphorus. All of these limitations of the claims must be considered regardless of whether or not they were supported by the specification as filed. In re Wilson, 57 CCPA 1029, 424 F.2d 1382, 165 USPQ 494 (1970); In re Miller, 58 CCPA 1182, 441 F.2d 689, 169 USPQ 597 (1971).

Claims 1 through 6 have been finally rejected under the first paragraph of 35 U.S.C. 112 both for lack of enablement and lack of description. We shall affirm the rejection based on lack of description in the specification as filed.

The examiner has not explained the basis of the rejection for lack of enablement and we find none independently.

Despite appellants' arguments to the contrary, we agree with the examiner's position of record that the negative limitations recited in the present claims, which did not appear in the specification as filed, introduce new concepts and violate the description requirement of the first paragraph of 35 U.S.C. 112. In re Anderson, 471 F.2d 1237, 176 USPQ 331 (CCPA 1973). The examiner's distinctions between the present case and the prior decisions cited by appellants are correct and we adopt his position in that regard as our own. It might be added that the express exclusion of certain elements implies the permissible inclusion of all other elements not so expressly excluded. This clearly illustrates that such negative limitations do, in fact, introduce new concepts.

[1] The decision of the examiner is affirmed.

AFFIRMED

- End of Case -

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Unique Steroid Congeners for Receptor Studies[1]

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Abstract

To determine the hormone-dependence of a tumor, it is preferable to use highly specific radiolabeled ligands when available, since often more than one class of steroid hormone receptor is present in the tissue specimen, and interference from classes other than the one under study cannot be readily eliminated. In this study, we describe a simple *in vitro* system used to define the molecular requirements for a highly specific interaction between a steroid and the receptor corresponding to a single class of hormone. It is based on the use of homogenate or crude 105,000 × *g* supernatant prepared from the target organs considered as end points in routine biological potency tests and on the use of available radioligands not bound by plasma proteins (tags) to single out the receptors. For each receptor singled out in the target organ cytoplasm, the ability of over 700 molecules to decrease bound radioactivity was compared to that of the natural hormone (relative binding affinity) with the use of a dextran-coated charcoal technique to separate bound from unbound steroid. On the basis of the results on 81 molecules, presented in this study, the effect of various substituents on the affinity and specificity of the natural hormones was determined. Molecules interacting markedly with several receptors were submitted to X-ray crystallography in order to establish whether overlap between the various conformations of the natural hormone and of the test molecule might not partly account for lack of specificity.

Introduction

The determination of the hormone-dependence of tumors in order to gauge the pertinence of endocrine therapy is becoming increasingly complex as receptors of different hormone classes are identified in the same tissue. Both estrogen and progestin receptors are now evaluated routinely in single specimens of human breast tumors (14, 25, 41, 42, 68, 69) and of human endometrium (51); androgen (17, 37, 38, 46, 52, 71, 76) and glucocorticoid (18, 70) receptors would also appear to be present. Several teams have identified androgen, estrogen, and progestin-like binding components in human benign hyperplastic prostate and prostate cancer (1, 13, 16, 23, 77).

Many of these studies have been made possible by the use of synthetic radioligands to assay the receptors belonging to a particular hormone class, since the natural hormones possess several highly restrictive disadvantages. Estradiol, testosterone, and progesterone bind to specific contaminating plasma proteins such as SBP[3] or CBG (30, 78); dihydrotestosterone is degraded under *in vitro* incubation conditions (7, 34, 67), and progesterone forms a complex with the cytoplasmic receptor which dissociates so rapidly that an exchange assay using labeled progesterone is difficult (19).

For study of several receptors in a single tissue specimen, the receptor specificity of the chosen radioligand has to equal at least that of the endogenous ligand. This study will describe the screening system used in our laboratory to evaluate the hormonal profile (receptor specificity) of a test substance in an attempt to develop new potent drugs (55, 56, 60) and new synthetic ligands. The system is based on competitive binding to the steroid receptor present in the cytosol of the organ routinely used as an end point in biological activity tests, thus ensuring by this choice of material that the receptor under study is present in appreciable quantities and is, under normal circumstances, functional.

Mouse uterus was used for studying the estrogen receptor, since uterotrophic activity is often regarded as a measure of estrogenicity. The mouse was considered preferable to the rat since it has a far lower concentration of circulating EBP (45), which interferes little with the use of estradiol as a radioligand. Estradiol binds with high affinity to EBP (59) and also to SBP (78); the latter, however, is not present in the rat or in the mouse. In future experiments, estradiol will be replaced by R 2858 [moxestrol (11β-methoxy-19-nor-1,3,5(10)-pregnatrien-20-yne-3,17β-diol)], which is a potent estrogen not bound by EBP or SBP (53, 57, 61). Rabbit uterus was used to study the progestin receptor, since histological grading of the proliferation of the rabbit endometrium is considered one of the more sensitive tests of progestational activity. However, owing to the presence of contaminating CBG and to the very high dissociation rate of the progesterone receptor complex, R 5020 [promegestone(17,21-dimethyl-19-nor-4,9-pregnadiene-3,20-dione)] replaced progesterone as a ligand, since it binds minimally to CBG (47, 54) and forms a much more stable receptor complex (49). The androgen receptor was identified in rat prostate with R 1881 [metribolone (17β-hydroxy-17α-methyl-estra-4,9,11-trien-3-one)]; the use of this organ and of this species present few problems since there is no contaminating SBP in the rat (6) and since the prostate of the young rat apparently does not contain any detectable progestin receptor (7) to which, as will be seen below, R 1881 binds. The most difficult receptors to identify conclusively without misinterpretation were the mineralocorticoid and glucocorticoid receptors, since these are often present concurrently (20) and since most ligands which bind firmly to one of these receptors also bind, even if less firmly, to the other. We have used labeled aldosterone and dexamethasone to

[1] Presented at the John E. Fogarty International Center Conference on Hormones and Cancer, March 29 to 31, 1978, Bethesda, Md.
[2] To whom requests for reprints should be addressed.

[3] The abbreviations used are: EBP, estradiol-binding protein; CBG, corticosteroid-binding globulin; SBP, sex steroid-binding protein; DHT, dihydrotestosterone; DCC, dextran-coated charcoal; RBA, relative binding affinity.

identify these receptors in rat kidney and liver, but are aware that these experimental conditions are not ideal, especially since the liver enzymes seem to remain operative under our *in vitro* incubation conditions. At present, all new test substances are being screened for glucocorticoid binding on the thymus, in which metabolism is less marked (15, 50).

For our screening system we have attempted to develop the simplest and "purest" models available, while eliminating the influence of plasma contamination by choosing an appropriate species or ligand and eliminating the influence of other binders by choosing a ligand that is as specific as possible for the receptor under study. Nevertheless, we cannot pretend that this system is ideal. According to available data in the literature, mouse uterus contains estrogen and progestin (19, 48, 49) receptors; rabbit uterus contains progestin, glucocorticoid (21), and estrogen (39) receptors; and rat prostate contains androgen, estrogen (72), and glucocorticoid (63) receptors. These receptors are present in variable concentrations. If the chosen radioligands are not totally receptor specific, in spite of all the precautions taken there will remain some doubt as to whether the receptor identified is really present in the tissue or whether the binding observed is not due to the presence of another unsuspected receptor (62) to which the ligand also binds. A distinction among the various receptors can be made then only by analyzing the differences in the kinetics of the binding of the radioligand to the individual receptors and by choosing incubation conditions which favor binding to one receptor rather than another.

To evaluate binding, a DCC adsorption method was used, since this has the advantage of leading to the dissociation of all very low-affinity specific binding. Several concentrations of a steroid were placed in competition with the radioligand, the displacement of bound radioligand was measured, and from these measurements binding curves were constructed. The results for 81 test substances, many of which have been synthesized by chemists at the Roussel-Uclaf Research Centre (2, 3, 11, 44, 73–75), follow.

Materials and Methods

Radioligands. The radioligands used in the experiments described in this paper are listed in Table 1.

Biological Activity Determination. Potency was evaluated in routine biological tests in the following manner: (*a*) Uterine weight was measured after 3-day s.c. administration to immature female mice. (*b*) Prostate weight was measured after 10-day s.c. administration to 3-week-old castrated male rats. (*c*) Endometrial proliferation was evaluated histologically after 5-day s.c. administration to estradiol-primed (5 μg estradiol per day for 5 days) immature rabbits.

Binding to Specific Plasma Proteins. Specific binding to SBP and to CBG was measured on human plasma after separation of the proteins by ammonium sulfate precipitation. Plasma from a pregnant woman (in her eighth month of pregnancy) was treated with 10% (v/v) DCC (6.25% dextran T80:3.125% charcoal Norit A) to remove unbound hormone and then with 42% $(NH_4)_2SO_4$ to precipitate SBP. After addition of the $(NH_4)_2SO_4$ and after magnetic stirring, the mixture was left overnight at 4°. The precipitate formed was centrifuged at 22,000 × *g* for 20 min and taken up in 10 mM Tris HCl (pH 7.4), 0.25 M sucrose buffer (final dilution, 1/100; protein concentration, 0.2 mg/ml). The supernatant was treated with 50% $(NH_4)_2SO_4$ to precipitate CBG according to a similar method (final dilution, 1/50; protein concentration, 0.12 mg/ml).

Specific binding to SBP was measured by DCC adsorption. Aliquots of 125 μl of SBP precipitate were incubated for 2 hr at 0° with 5 nM [^{3}H]DHT in the presence of increasing concentrations (10 to 2500 nM) of unlabeled competitor. A DCC suspension (100 μl; 0.625% dextran T80:1.25% charcoal Norit A) were added to 100 μl of incubate in a microtiter plate (Greiner plates, M220-24Å; System Cooke) and shaken for 10 min at 0°. After centrifugation for 10 min at 800 × *g*, the radioactivity of a 100-μl supernatant aliquot was counted. The percentage bound [^{3}H]DHT was plotted against the concentration of unlabeled competitor in the tube, and the competitor concentration required for 50% displacement of [^{3}H]DHT from its specific binding sites was determined. Results are expressed as the ratio of the DHT concentration to competitor concentration for 50% displacement.

Specific binding to CBG was measured by equilibrium dialysis. Aliquots of 0.5 ml of CBG precipitate were dialyzed against 15 ml of [^{3}H]hydrocortisone (2.5 nM) in Tris-sucrose buffer in the presence of various concentrations (2.5 to 2500 nM) of unlabeled competitor. After magnetic stirring at 0–4° for 48 hr, the radioactivity of 0.2-ml samples from inside and outside the dialysis membrane (Union Carbide Corp., Chicago, Ill.) was measured. The percentage of decrease in bound radioactive steroid was determined, and the competitor concentration giving rise to a 50% decrease was deduced.

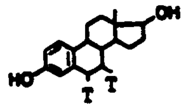
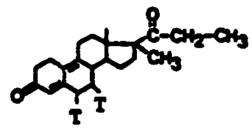
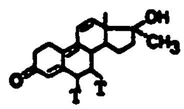
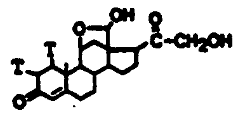
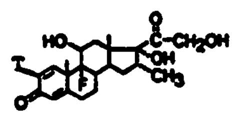
Cytosol Preparation and Incubation. Cytosols were prepared by centrifuging homogenates obtained from the organs of various species as indicated in Table 2 and incubated with the corresponding radioligand, *i.e.*, unless otherwise stated, 5 nM [^{3}H]estradiol was incubated for 2 hr at 0° with mouse uterus cytosol to label the estrogen receptor, 2.5 nM [^{3}H]R 5020 was incubated for 2 hr at 0° with rabbit uterus cytosol to label the progestin receptor, 2.5 nM [^{3}H]R 1881 were incubated for 2 hr at 0° with rat prostate cytosol to label the androgen receptor, 5 nM [^{3}H]dexamethasone were incubated for 4 hr at 0° with rat liver cytosol to label the glucocorticoid receptor, and 2.5 nM [^{3}H]aldosterone were incubated for 30 min at 25° with rat kidney homogenates, which were then centrifuged at 800 × *g* for 10 min at 0° to label the mineralocorticoid receptor. All incubations were performed in the absence and presence of 0 to 2500 nM unlabeled competing steroid.

Bound Steroid Measurement by DCC Adsorption. A 100-μl aliquot of incubated cytosol was stirred for 10 min at 0–4° with 100 μl DCC (0.625% dextran 8C, 000:1.25% charcoal Norit A) in a microtiter plate and then centrifuged for 10 min at 800 × *g*. The radioactivity of a 100-μl supernatant sample was measured.

RBA Determinations. The percentage of radioligand bound in the presence of competitor compared to that bound in its absence was plotted against the concentration of unlabeled competing steroid. A standard curve for the competition of unlabeled radioligand was constructed with

Table 1

Radioligands used to label steroid hormone receptors in our screening system

Radioligand[a]	Formula	Specific activity (Ci/mmol)	Thin-layer chromatography solvent systems[b]
[6,7-^{3}H]Estradiol		50	Benzene:ethyl acetate (1:1, v/v) Methylene chloride:methanol (9:1, v/v)
[6,7-^{3}H]R 5020		57	Benzene:ethyl acetate (7:3, v/v)
[6,7-^{3}H]R 1881		56	Benzene:ethyl acetate (5:5, v/v)
[1,2-^{3}H]Aldosterone		53	Benzene:ethanol (9:1, v/v)
[2-^{3}H]Dexamethasone		26	Carbon tetrachloride:acetone (6:4, v/v)

[a] All the radioligands were synthesized by the Roussel-Uclaf Research Centre.
[b] Prior to use, radiochemical purity was checked by thin-layer chromatography with the use of Merck silica gel plates. All of the radioligands were more than 98% pure.

Table 2

Preparation of cytosols used to study steroid hormone receptors in our screening system

Species[a] and strain	Status	Organ	Buffer	Homogenization tissue:buffer ratio (w/v)	Cytosol preparation
Mouse (Swiss)	Female (18 days old)	Uterus		1:50	
Rabbit (New Zealand)	Female (50–55 days old, estrogen-primed)[b]	Uterus	TS buffer[c]	1:50	Centrifugation of homogenate at 105,000 × *g* for 60 min at 4°
Rat (Sprague-Dawley)	Male (140–160 g castrated for 24 hr)	Prostate		1:5	
	Male (140–160 g adrenalectomized for 4–7 days)	Perfused kidney	Krebs-Ringer phosphate buffer	1:3	
		Perfused liver	TS buffer	1:10	105,000 × *g* for 60 min at 4°

[a] All animals were supplied by Iffa-Credo, France, except for the rabbits, which were obtained from Elevage Cunicole, Chatillon-Coligny, France.
[b] The rabbits were primed with 25 μg of estradiol in ethanol applied to the dorsal skin and were killed after 4 days.
[c] TS buffer, 10 mM Tris-HCl (pH 7.4): 0.25 M sucrose.

the use of 9 to 10 concentrations; 5 or 6 concentrations of each competitor were tested. These were chosen to provide a linear portion on a semilog plot which would cross the point of 50% competition. From this plot, the molar concentrations of unlabeled radioligand or steroid competitor that reduced radioligand binding by 50% were determined. The effectiveness of a competitor was established with the use of the ratio of unlabeled radioligand concentration for 50% competition to competitor concentration for 50% competition. This ratio was multiplied by 100 and termed the RBA. The RBA's of the endogenous hormones and of dexamethasone were taken to be equal to 100.

Table 3
Chemical names and trivial names of the test substances

A1	Pregn-4-ene-3,20-dione (progesterone)
A2	5α-Pregna-3,20-dione
A3	5β-Pregna-3,20-dione
A4	3β-Hydroxy-5α-pregn-20-one
A5	3β-Hydroxy-5β-pregn-20-one
A6	3α-Hydroxy-5α-pregn-20-one
A7	3α-Hydroxy-5β-pregn-20-one
A8	19-Norpregn-4-ene-3,20-dione (19-norprogesterone)
A9	19-Norpregna-4,9-diene-3,20-dione
A10	19-Norpregna-4,9,11-triene-3,20-dione
A11	2,2-Dimethyl-19-norpregna-4,9-diene-3,20-dione
A12	16α-Methyl-19-norpregn-4-ene-3,20-dione
A13	16,16-Dimethylpregn-4-ene-3,20-dione
A14	6α,16α-Dimethylpregn-4-ene-3,20-dione
A15	17α-Methyl-19-norpregn-4-ene-3,20-dione
A16	17α-Methyl-19-norpregna-4,9-diene-3,20-dione
A17	17α-Methyl-19-norpregna-4,9,11-triene-3,20-dione
A18	17α,21-Dimethyl-19-norpregna-4,9-diene-3,20-dione (promegestone)
A19	11β-Hydroxypregn-4-ene-3,20-dione
A20	11α-Hydroxypregn-4-ene-3,20-dione
A21	11β-Hydroxy-19-norpregna-4,9-diene-3,20-dione
A22	11β-Methoxy-19-norpregna-4,9-diene-3,20-dione
A23	17α-Hydroxypregn-4-ene-3,20-dione
A24	17α-Hydroxy-19-norpregna-4,9-diene-3,20-dione
B1	17β-Hydroxy-androst-4-en-3-one (testosterone)
B2	17β-Hydroxy-5α-androstan-3-one (dihydrotestosterone)
B3	17β-Hydroxy-5β-androstan-3-one
B4	5α-Androstane-3β,17β-diol
B5	5α-Androstane-3α,17β-diol
B6	17β-Hydroxyestr-4-en-3-one (nortestosterone)
B7	17β-Hydroxyestra-4,9-dien-3-one
B8	17β-Hydroxyestra-4,9,11-trien-3-one
B9	13β-Ethyl-17β-hydroxygon-4-en-3-one
B10	17β-Hydroxy-13β-propylgon-4-en-3-one
B11	17β-Hydroxy-17α-pregn-4-en-20-yn-3-one
B12	17β-Hydroxy-19-nor-17α-pregn-4-en-20-yn-3-one (norethindrone)
B13	17β-Hydroxy-19-nor-17α-pregna-4,9-dien-20-yn-3-one
B14	17β-Hydroxy-19-nor-17α-pregna-4,9,11-trien-20-yn-3-one (norgestrienone)
B15	13β-Ethyl-17β-hydroxy-18,19-dinor-17α-pregn-4-en-20-yn-3-one (norgestrel)
B16	17β-Hydroxy-13β-propyl-18,19-dinor-17α-pregn-4-en-20-yn-3-one
B17	13β-Ethyl-17β-hydroxy-18,19-dinorpregna-4,9,11-trien-20-yn-3-one (gestrigone)
B18	17β-Hydroxy-13β-propylgona-4,9,11-trien-3-one
B19	17β-Hydroxy-13β-propyl-18,19-dinor-17α-pregna-4,9,11-trien-20-yn-3-one
B20	17β-Hydroxy-2α-methylestr-4-en-3-one
B21	17β-Hydroxy-17α-methylestr-4-en-3-one (methylestrenolone)
B22	17β-Hydroxy-17α-methylestra-4,9-dien-3-one
B23	17β-Hydroxy-17α-methylestra-4,9,11-trien-3-one (methyltrienolone, metribolone)
B24	13β-Ethyl-17β-hydroxy-17α-methylgona-4,9,11-trien-3-one
B25	17β-Hydroxy-2β,17α-dimethylestra-4,9,11-triene-3-one
B26	17β-Hydroxy-2,2,17α-trimethylestra-4,9,11-trien-3-one
B27	17β-Hydroxy-7α,17α-dimethylestra-4,9-dien-3-one
B28	11β,17β-Dihydroxy-17α-pregn-4-en-20-yn-3-one
B29	11β,17β-Dihydroxy-19-nor-17α-pregna-4,9-dien-20-yn-3-one
B30	13β-Ethyl-11β,17β-dihydroxy-18,19-dinorpregna-4,9-dien-20-yn-3-one
B31	17β-Hydroxy-11β-methoxy-19-nor-17α-pregn-4-en-20-yn-3-one
B32	17β-Hydroxy-11β-methoxy-19-nor-17α-pregna-4,9-dien-20-yn-3-one
D1	21-Hydroxypregn-4-ene-3,20-dione (deoxycorticosterone)
D2	21-Hydroxy-5β-pregna-3,20-dione
D3	21-Hydroxypregna-4,6-diene-3,20-dione
D4	21-Hydroxy-2β-methylpregn-4-ene-3,20-dione
D5	21-Hydroxy-2α-methylpregn-4-ene-3,20-dione
D6	21-Hydroxy-2,2-dimethylpregn-4-ene-3,20-dione
D7	11β,21-Dihydroxypregn-4-ene-3,20-dione (corticosterone)
D8	17α,21-Dihydroxypregn-4-ene-3,20-dione (cortexolone)
D9	11β,17α,21-Trihydroxypregn-4-ene-3,20-dione (hydrocortisone)
D10	11β,21-Dihydroxypregna-1,4-diene-3,20-dione (1-dehydrocorticosterone)
D11	11β,17α,21-Trihydroxypregna-1,4-diene-3,20-dione (prednisolone)
E1	Estra-1,3,5(10)-triene-3,17β-diol (17β-estradiol)
E2	13β-Ethylgona-1,3,5(10)-triene-3,17β-diol

Table 3—*Continued*

E3	13β-Propylgona-1,3,5(10)-triene-3,17β-diol
E4	19-Nor-17α-pregna-1,3,5(10)-trien-20-yne-3,17β-diol (ethynyl estradiol)
E5	16β-Ethynylestra-1,3,5(10)-triene-3,16α-diol
E6	2-Methylestra-1,3,5(10)-triene-3,17β-diol
E7	7α-Methylestra-1,3,5(10)-triene-3,17β-diol
E8	17α-Methylestra-1,3,5(10)-triene-3,17β-diol
E9	Estra-1,3,5(10)-triene-3,11β,17β-triol
E10	11β-Methoxyestra-1,3,5(10)-triene-3,17β-diol
E11	11α-Methoxyestra-1,3,5(10)-triene-3,17β-diol
E12	11β-Methoxyestra-1,3,5(10)-triene-3,16α,17β-triol
E13	11β-Methoxy-19-nor-17α-pregna-1,3,5(10)-trien-20-yne-3,17β-diol(moxestrol)
E14	11α-Methoxy-19-nor-17α-pregna-1,3,5(10)-trien-20-yne-3,17β-diol

In order to be able to readily compare the results for the 81 competing steroids listed in Table 3, the exact numerical values of RBA's have not been given (except for the radioligands themselves and for the endogenous hormones). Instead, the RBA's for the 5 different receptors (estrogen, progestin, androgen, mineralocorticoid, and glucocorticoid) have been graded according to the scale illustrated in Chart 1. The results given are the means of at least 2 determinations which differ by less than 15%.

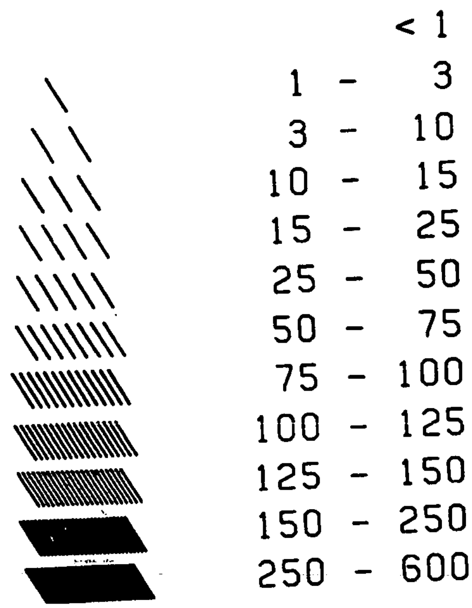


Chart 1. Grading used to represent RBA in Charts 5 to 8.

Results

I. Radioligand Profile

Biological Activity. All the synthetic hormones we have radiolabeled for the detection of steroid hormone receptors are highly potent pharmacologically in routine biological tests. As shown in Chart 2, the estrogen R 2858 is approximately 5–10 times as potent as estradiol in increasing the weight of mouse uterus, the androgen R 1881 is about 50 times as potent as testosterone in increasing the weight of rat prostate, and the progestin R 5020 is about 50 times as potent as progesterone in inducing endometrial proliferation in the estrogen-primed rabbit. The biological activity of R 2858 has been included [although it is not the radioligand used in the screening system, it now frequently replaces estradiol for the detection and assay estrogen receptors in human breast tumors because of its lack of binding to SBP and the slow dissociation rate of the complex that it forms with the estrogen receptor (58, 61)].

Binding to Specific Plasma Proteins. Data on the lack of specific binding of the chosen radioligands to plasma proteins specific for the endogenous hormones have already been published for several species (12, 27, 54, 61, 62). Only the species used in the screening system and humans (since the ultimate aim of the development of these radioligands is their use for receptor assay in normal and neoplastic human tissues) concern us here. As regards the species used in the screening system, R 5020 does not bind to CBG in rabbit plasma (47) and dexamethasone does not bind to CBG in rat plasma. There is little EBP (45) and no SBP in mouse plasma, thus permitting the use of labeled estradiol, and there is no SBP in rat plasma rendering any binding of R 1881 to SBP irrelevant. In the case of human plasma, as shown in Chart 3 and Table 4, whereas all the natural hormones compete appreciably either for binding to SBP, measured by a DCC adsorption method, or to CBG, measured by equilibrium dialysis, none of the synthetic steroids exhibit significant binding. Furthermore, the R 5020 binding which is recorded to CBG by equilibrium dialysis is totally dissociated in the presence of DCC in our screening conditions.

Binding to Specific Tissue Receptors. The relative concentrations of test substances required to displace 50% of specifically bound radioactivity were determined as described in "Materials and Methods" and as illustrated in Chart 4. The binding profile of the radioligands compared to the natural hormones is given in Table 5, which yields 2 kinds of information. It shows whether an interaction occurs between the steroid and receptor (results obtained after short incubation times), and it indicates whether the complex formed is stable (comparison with results obtained after long incubation times and/or higher temperatures) (9). (In the forthcoming competition results on the 81 test steroids, only the results for short incubation times are given, since to choose a suitable ligand for labeling, it is necessary to know whether any interaction occurs with a particular receptor). According to the data in Table 5, R 2858 is as specific as estradiol. It binds less than estradiol to the progestin and androgen receptors, but more than estradiol to the glucocorticoid receptor. Its binding to the estrogen receptor itself is under certain conditions comparable, if not stronger, than that of estradiol; in fact, at 0° in mouse uterine cytosol R 2858 associates 5 to 10 times slower with the estrogen receptor than with estradiol, but forms a complex which, at 25°, dissociates about 5 times

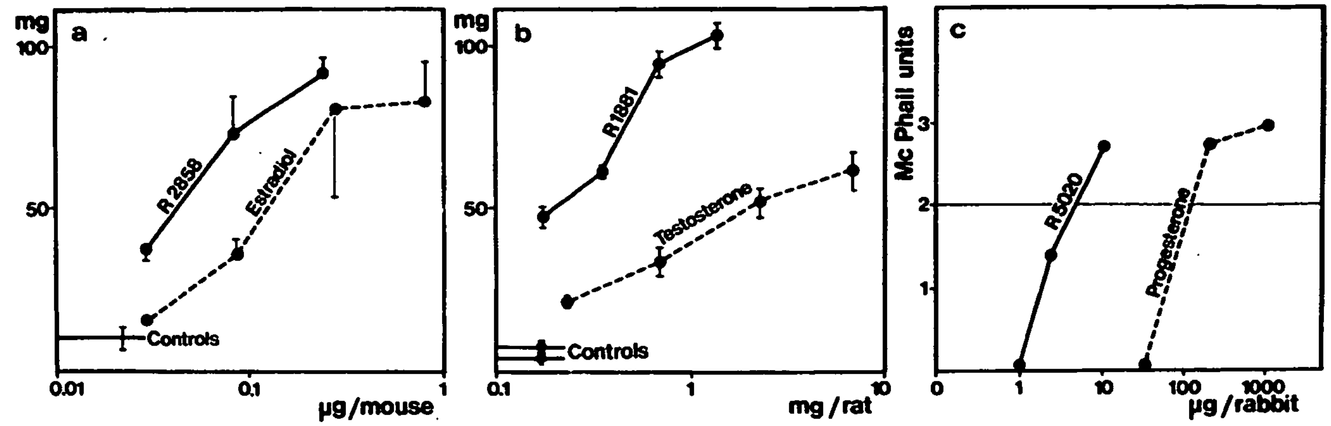


Chart 2. Potency of some of the synthetic hormones used to label steroid receptors. In *a*, uterine weight was measured after 3-day s.c. administration to immature mice. In *b*, prostate weight was measured after 10-day s.c. administration to 3-week-old castrated rats. In *c*, endometrial proliferation was measured after 5-day s.c. administration to estradiol-primed rabbits. In *a* and *b*, the total dose administered has been plotted; in *c*, the daily dose.

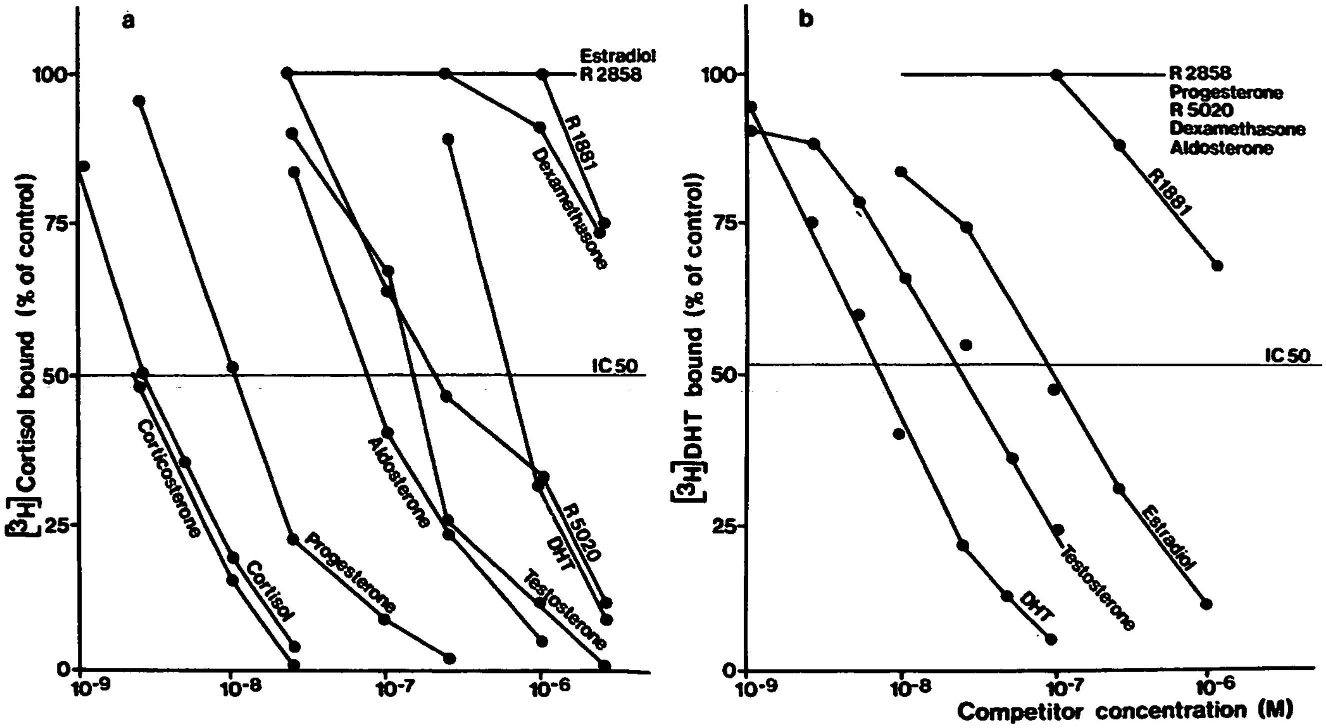


Chart 3. Binding to semipurified sex SBP labeled with [^{3}H]DHT and CBG labeled with [^{3}H]cortisol.

slower. R 5020 binds much more firmly than does progesterone to the progestin receptor. It competes very little, less than progesterone, for binding to the androgen and mineralocorticoid receptors, but more than progesterone for binding to the glucocorticoid receptor. R 1881 is highly unspecific, since it binds to a similar extent to the progestin and androgen receptors and also binds appreciably, compared to testosterone, to the mineralo- and glucocorticoid receptors. Dexamethasone not only binds more strongly to the glucocorticoid receptor than do cortisol and corticosterone, but is also more specific than corticosterone, since it competes less for progestin and mineralocorticoid binding.

II. In Vitro Screening

Functional Groupings. Several studies have established the importance of certain functional groupings on the steroid nucleus for binding to steroid hormone receptors. Thus, compounds which bind effectively to the estrogen receptor possess an aromatic A ring and 2 hydroxy groups, in positions C-3 and C-17, separated by about 12.2 Å (24, 26). Blocking the C-3 and/or C-17 hydroxyl by methylation invariably results in loss of binding affinity (24, 28, 33, 56, 57). Alteration of the position of the phenolic hydroxy group of β-estradiol from the C-3 to the C-2 position reduces RBA to about one-third of that of β-estradiol (65).

A 3-keto-4-ene structure is a common feature for the effective binding of a ligand to the progestin, androgen, mineralocorticoid, and glucocorticoid receptors. If the double bond of progesterone (A1) is reduced (Chart 5) to give a 3-keto-5α structure (A2) or a 3-keto-5β structure (A3), the RBA is decreased considerably and much more so in the

Table 4

Binding to specific proteins in human plasma

The RBA's of compounds that did not compete at a 2500 nM concentration have been given as <0.2 for binding to SBP (since the IC_{50} of DHT is 5 nM) and as <0.1 for binding to CBG (since the IC_{50} of cortisol is 2.5 nM).

	SBP	CBG
Estradiol	8.7	<0.1
R 2858 (moxestrol)	<0.2	<0.1
Progesterone	<0.2	25
R 5020 (promegestone)	<0.2	0.9
Testosterone	26	3
5α-DHT	$\boxed{100}$	0.8
R 1881 (metribolone)	0.2	<0.1
Aldosterone	<0.2	6.0
Cortisol	<0.2	$\boxed{100}$
Corticosterone	<0.2	107
Dexamethasone	<0.2	<0.1

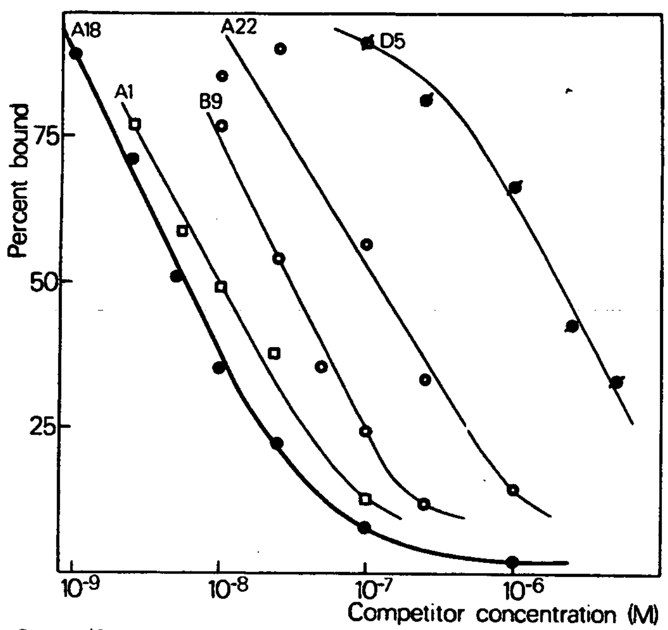


Chart 4. Competitive binding curves of several progestins in rabbit uterus cytosol. Radioligand and competitor were incubated with diluted cytosol for 2 hr at 0°. Bound steroid was measured by a DCC adsorption technique and plotted against the concentration of unlabeled steroid in the tube. The concentrations of unlabeled radioligand and competing steroid required to decrease the binding measured in the absence of competitor by 50% were determined. Their ratio (times 100) give the relative binding affinity.

latter than in the former case (31, 32, 35, 40, 56). Reduction of the double bond of testosterone (B1) gives 5α-DHT (B2), which has a slightly higher affinity than testosterone or 5β-dihydrotestosterone (B3), which has virtually no affinity at all (64). 5β reduction of deoxycorticosterone (D1) gave a totally inactive molecule (D2).

Binding of all these molecules is also decreased on reducing the 3-keto group, *e.g.*, A4 and A6 compared to A2, A5 and A7 compared to A3 (31, 40, 56), B4 and B5 compared to B2 (22, 36, 64), or in the absence of a grouping in C-3 (56). The substituent in position C-17 seems to be of less importance since, for instance, compounds which bind to the progestin receptor may possess at this position either an acetyl grouping or a β-hydroxy group in the presence of an α-methyl or ethynyl. Even on reduction of the acetyl group, binding affinity may be maintained as long as the 21 hydroxy group is in the β and not in the α configuration (31, 32, 35, 55, 66).

On the basis of the above observations, it would appear that in order to select a high-affinity specific binder, it is preferable not to tamper with the functional groups and to screen only molecules where these groups have been left free for binding to the relevant receptor. By substituting at other positions of the steroid nucleus, binding to this receptor might be enhanced, hopefully without the introduction of other subsidiary binding components, thus paving the way to the design of new ultraspecific ligands.

In the following charts (5 to 8), the binding profiles of 81 molecules are given. This selection of molecules is complementary to a first series (60) and illustrates the effect of unsaturation, chain length, ethynylation, methylation, and hydroxylation on the natural hormones.

Removal of C-19 Methyl, Unsaturation, and Homologation of C-13 Methyl. As indicated in Chart 5, the removal of the C-19 methyl group of both progesterone- and testosterone-enhanced binding to their respective receptors (A8 compared to A1 and B6 compared to B1). In both cases, however, this modification led to the appearance of slightly more marked binding to a subsidiary receptor [the mineralocorticoid receptor in the case of norprogesterone (A8) and the progestin receptor in the case of nortestosterone (B6)]. Binding to the parent receptor was similarly enhanced by the introduction of double bonds in Δ9 (A9 and B7) or in Δ9,11 (A10 and B8), but these also affected subsidiary binding. The triene A10, a derivative of progesterone, bound appreciably to the androgen receptor, and the triene B8, a derivative of testosterone, bound even more appreciably to the progestin receptor. The introduction of these double bonds would thus appear to induce a certain lack of specificity, which will be confirmed below. Only one example of the introduction of a double bond, in Δ6, is illustrated for the corticoid structure (D3). This molecule has lost some affinity for the mineralocorticoid and progestin receptors and binds weakly to the glucocorticoid receptor.

Chart 5 also illustrates the effect of homologation of the C-13 methyl in the testosterone and estradiol series. In both series, this modification resulted in a slight decrease in binding affinity for the parent receptor. This decrease was more marked for the estradiol derivatives (E2 and E3 compared to E1) than for the testosterone derivatives (B9 and B10 compared to B6). There was no appreciable effect on the secondary receptor (progestin receptor for the testosterone series and androgen receptor for the estradiol series), but in both series the presence of very slight glucocorticoid binding was noted.

Ethynylation. A study of 8 17α-ethynylated derivatives in the testosterone series conclusively established that the introduction of this substituent into the testosterone or nortestosterone molecule is associated with reduced binding to the androgen receptor and increased binding to the progestin receptor. These effects are clearly seen upon comparison of pairs of molecules [B1 and B11, B6 and B12,

Table 5

RBA's for steroid hormone receptors

	Estrogen receptor		Progestin receptor		Androgen receptor (2 hr, 0°)	Mineralocorticoid receptor (30 min, 25°)	Glucocorticoid receptor	
	2 hr, 0°	5 hr, 25°	2 hr, 0°	24 hr, 0°			(4 hr, 0°)	(20 hr, 0°)
Estradiol	$\boxed{100}$	$\boxed{100}$	2.6 ± 0.9	0.9 ± 0.2	7.9 ± 1.3	0.13 ± 0.03	0.6 ± 0.1	0.15
R 2858 (moxestrol)	12 ± 1[a]	112 ± 8	0.8 ± 0.3	0.7	<0.1	<0.1	3.2 ± 0.4	0.8
Progesterone	<0.1		$\boxed{100}$	$\boxed{100}$	5.5 ± 0.6	8.0 ± 1.0	0.24 ± 0.07	0.1
R 5020 (promegestone)	<0.1		222 ± 7	530 ± 30	1.2 ± 0.5	0.3 ± 0.1	14 ± 2	3.6 ± 0.8
Testosterone	<0.1		1.2 ± 0.3	1.1 ± 0.3	$\boxed{100}$	0.9 ± 0.1	0.17 ± 0.22	<0.1
5α-DHT	<0.1		1.4 ± 0.7	0.9	60 ± 3	0.15	<0.1	
R 1881 (metribolone)	<0.1	208 ± 18		191 ± 28	199 ± 4	18 ± 2	26 ± 5	6.1 ± 0.3
Aldosterone	<0.1		1.1 ± 0.2	0.8	<0.1	$\boxed{100}$	2.7 ± 1.2	0.13 ± 0.03
Cortisol	<0.1		<0.1		<0.1	14 ± 0.6	40 ± 7	5 ± 2
Corticosterone	<0.1		5.1 ± 1.0	2.8 ± 0.3	0.5 ± 0.2	33 ± 5	23 ± 6	0.2 ± 0.1
Dexamethasone	<0.1		0.4 ± 0.1	0.1	0.5	17 ± 2	$\boxed{100}$	$\boxed{100}$

[a] Mean ± SE of either 3 or more experiments or of 2 experiments giving results that differ by less than 15%.

B7 and B13, B8 and B14, B9 and B15, B10 and B16, B18 and B19 (Chart 6)]. At times, the effect was so marked that the 17α-ethynyl-substituted testosterone derivatives bound more to the progestin than to the androgen receptor. Many are potent progestins.

Previous observations on unsaturation and C-13 methyl homologation can also be confirmed and extended on comparing the ethynylated derivatives. Binding of the triene (B14) to the androgen receptor was more marked than that of the corresponding monoene (B12) and diene (B13); however, the diene competed less than did the monoene. This last result is in accord with observations on the nonethynylated derivatives; namely, B7 bound less than B8. The lack of specificity of trienic structures was also confirmed, insofar as the triene B14 competed slightly more than did the diene B13 for the progestin receptor, an observation in analogy with the results for B8 and B7. As regards homologation of the C-13 methyl, it would appear that the secondary binding most closely associated with a C-13 ethyl group might be corticoid binding, since it distinguishes B15 from B12, B17 from B14, and B9 from B6. This seemed, however, characteristic of the ethyl group and did not hold true for the propyl group.

In the estradiol series, 17α-ethynylation also introduced progestin binding and furthermore enhanced binding to the estrogen receptor, although the latter is not readily apparent in Chart 5 because of the lack of sensitivity of the grading (E4 compared to E1). The compound E5, in which the ethynyl (β) and hydroxyl (α) groups have been transferred from position 17 to 16, has no binding affinity at all for the estrogen receptor.

Methylation. Chart 7 illustrates a few examples of the effect of methylation on the binding profiles of the natural hormones.

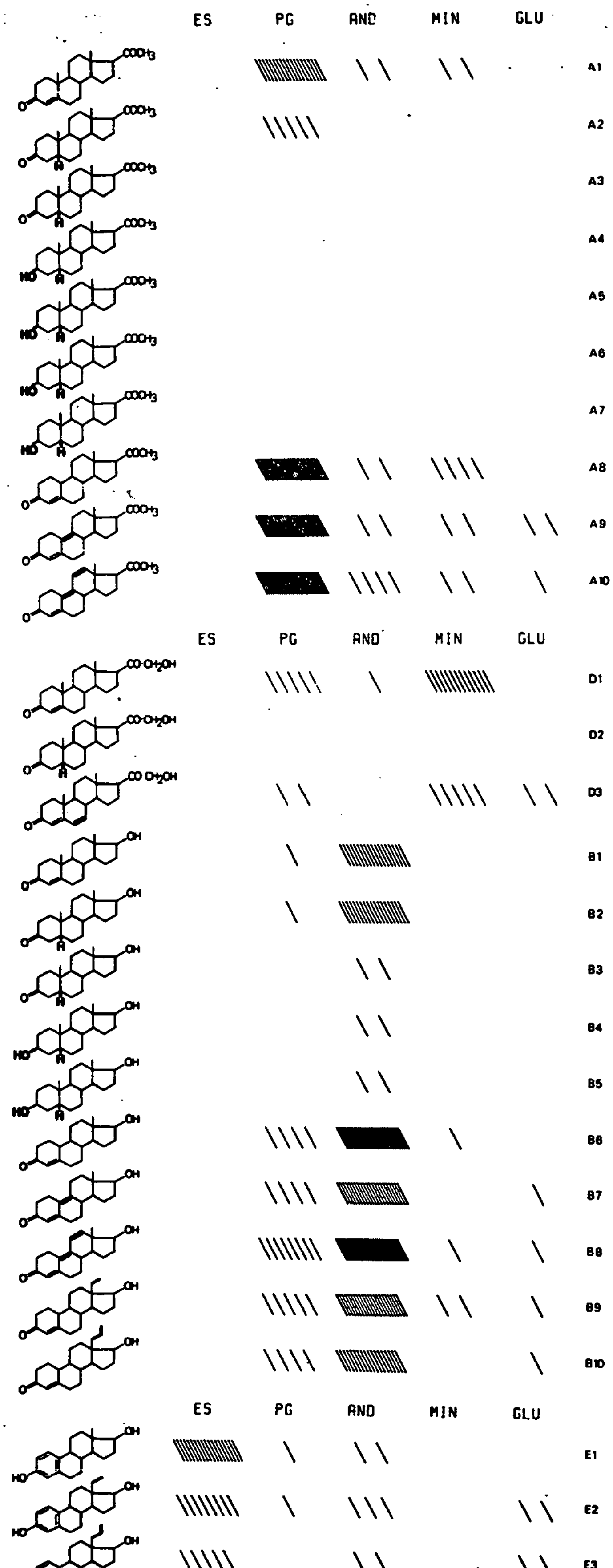
Whether in the testosterone, corticosteroid, or estradiol series, methylation in position C-2 decreased binding to the corresponding receptor (B20 compared to B6, D4 and D5 compared to D1, and E6 compared to E1). The presence of a gem-dimethyl group in C-2 even further decreased binding. A11 had no affinity whatsoever for a steroid hormone receptor, and D6 was also virtually totally inactive.

Three C-16-substituted methyl derivatives were compared in the progesterone series. 16α-Methyl progesterone (A12) bound less than did progesterone, and the 16-*gem*-dimethyl derivative (A13) bound even less. When the second methyl substituent was introduced at position C-6 (A14), instead of C-16 (A13), progestin binding was similarly decreased and, furthermore some glucocorticoid binding was introduced (A14).

The effect of 17α-methylation could be compared in the progesterone, testosterone, and estradiol series. This substituent could be tentatively associated with progestin binding, since all 3 17α-substituted compounds (A15, B21, and E8) bound more markedly to the progestin receptor than did the corresponding unsubstituted compounds (A8, B6, and E1, respectively). This substituent slightly decreased binding to the androgen receptor (B21 compared to B6) and noticeably decreased the affinity of estradiol for the estrogen receptor (E8 compared to E1). As expected, the further introduction of C-2 methyl substituents into 17α-methyl testosterone derivatives (B25 and B26 compared to B23) considerably decreased binding to the androgen and progestin receptor (5, 34). The further introduction of a C-7 methyl substituent had no effect on androgen binding and slightly increased progestin binding (B27 compared to B22).

Chart 7 also yields further information on unsaturation; in particular it reveals that the lack of specificity induced by unsaturation is greater in the testosterone (B21, B22, and B23) than in the progesterone (A15, A16, and A17) series, and on C-13 methyl homologation, insofar as the C-13 ethyl derivative B24, like other ethyl derivatives, exhibits significant glucocorticoid binding.

Hydroxylation. Derivatives hydroxylated in position C-11 were available in all 4 series (Chart 8). In the progesterone and corticosteroid series, the 11β-OH group led to a decrease in progestin and mineralocorticoid binding and to the introduction of glucocorticoid binding (A19 compared to A1 and D7 compared to D1). A similar but less-marked effect was observed in the testosterone series [B28 (Chart 8) compared to B11 (Chart 6)]. 11β-Hydroxylation of estradiol led to a very poor estrogen binder (E9). When there was a double bond present in Δ9, the decrease in binding on 11β-hydroxylation was even more pronounced [e.g., A21 and



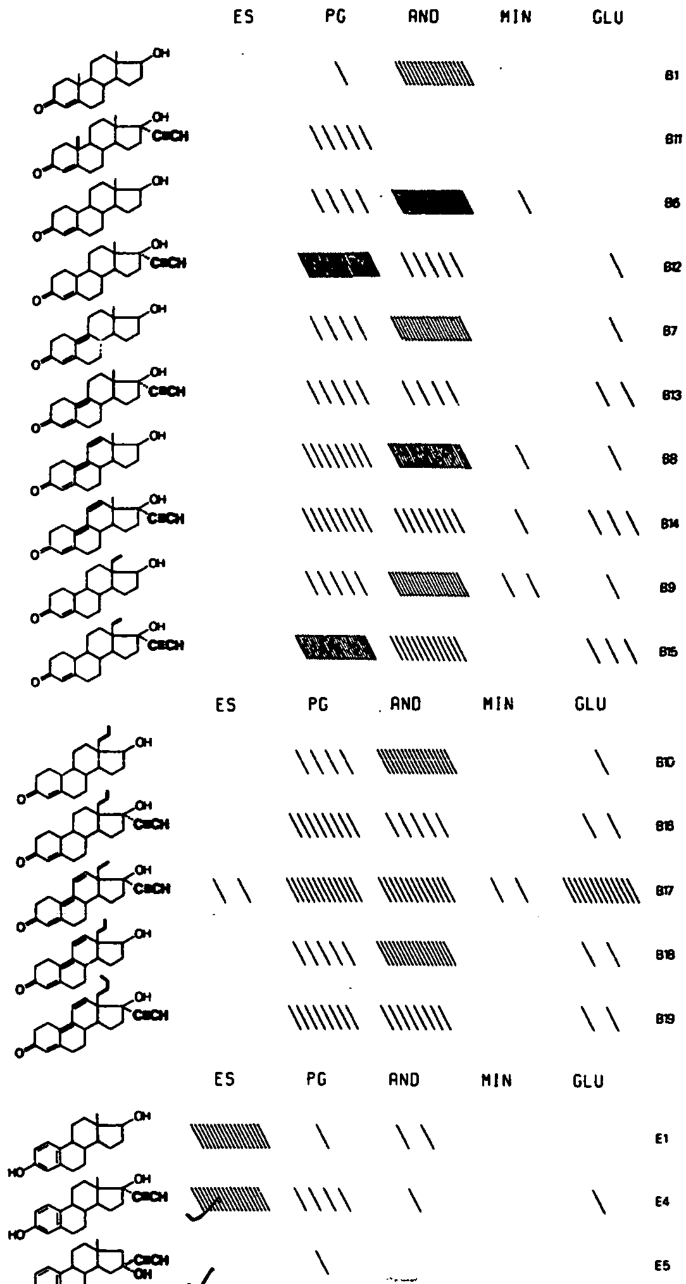


Chart 6. Effect of ethynylation on the binding profiles of testosterone and estradiol to the estrogen (*ES*), progestin (*PG*), androgen (*AND*), mineralocorticoid (*MIN*) and glucocorticoid (*GLU*) receptors.

B29 compared to A9 and B13 (Chart 6)]. In the corticosteroid series, a double bond in Δ1 enhanced binding to the glucocorticoid receptor (D10 compared to D7).

Methoxylation of the 11β-hydroxyl substituent had different effects in the various series, *e.g.*, methoxylation of the 11β-hydroxy substituted dienes had little effect on the binding profiles (A22 and B32 compared to A21 and B29), whereas methoxylation of the monoene B28 increased pro-

Chart 5. Effect of unsaturation, of removal of a C-19 methyl, and of homologation of the C-13 methyl on the binding profiles of progesterone, testosterone, deoxycorticosterone, and estradiol to the estrogen (*ES*), progestin (*PG*), androgen (*AND*), mineralocorticoid (*MIN*), and glucocorticoid (*GLU*) receptors.

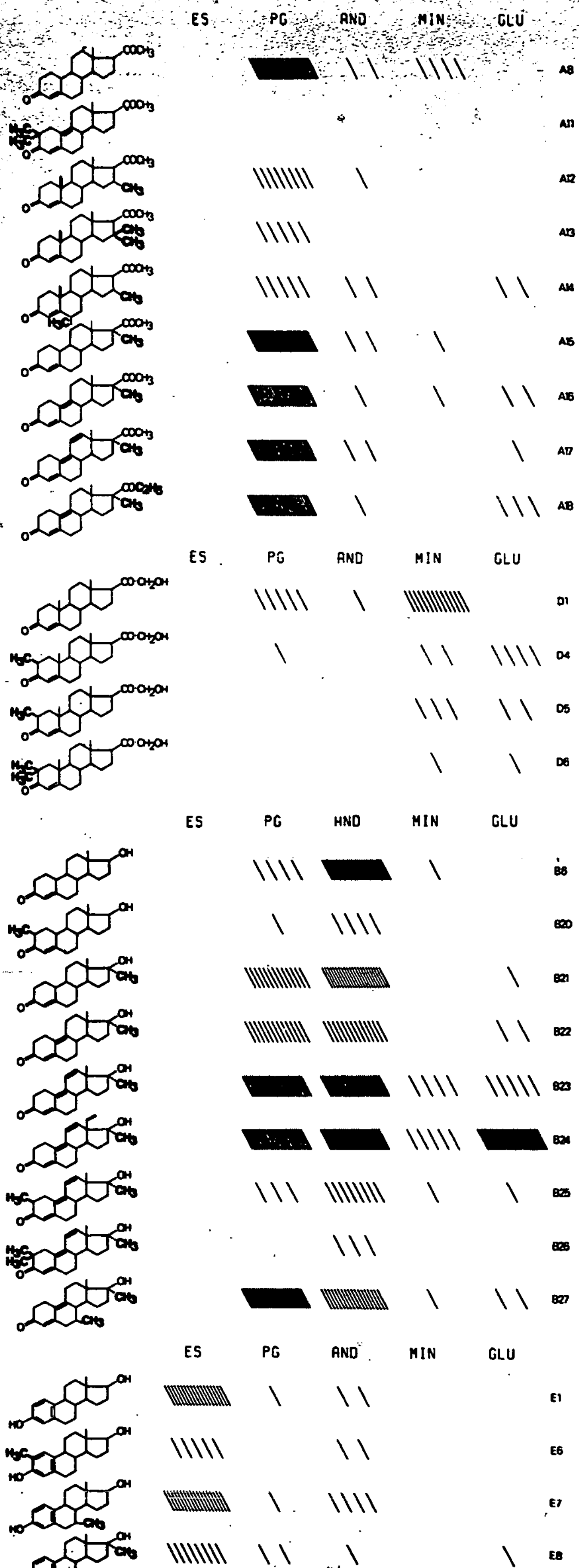


Chart 7. Effect of methylation on the binding profiles of progesterone, testosterone, deoxycorticosterone, and estradiol to the estrogen (*ES*), progestin (*PG*), androgen (*AND*), mineralocorticoid (*MIN*), and glucocorticoid (*GLU*) receptors.

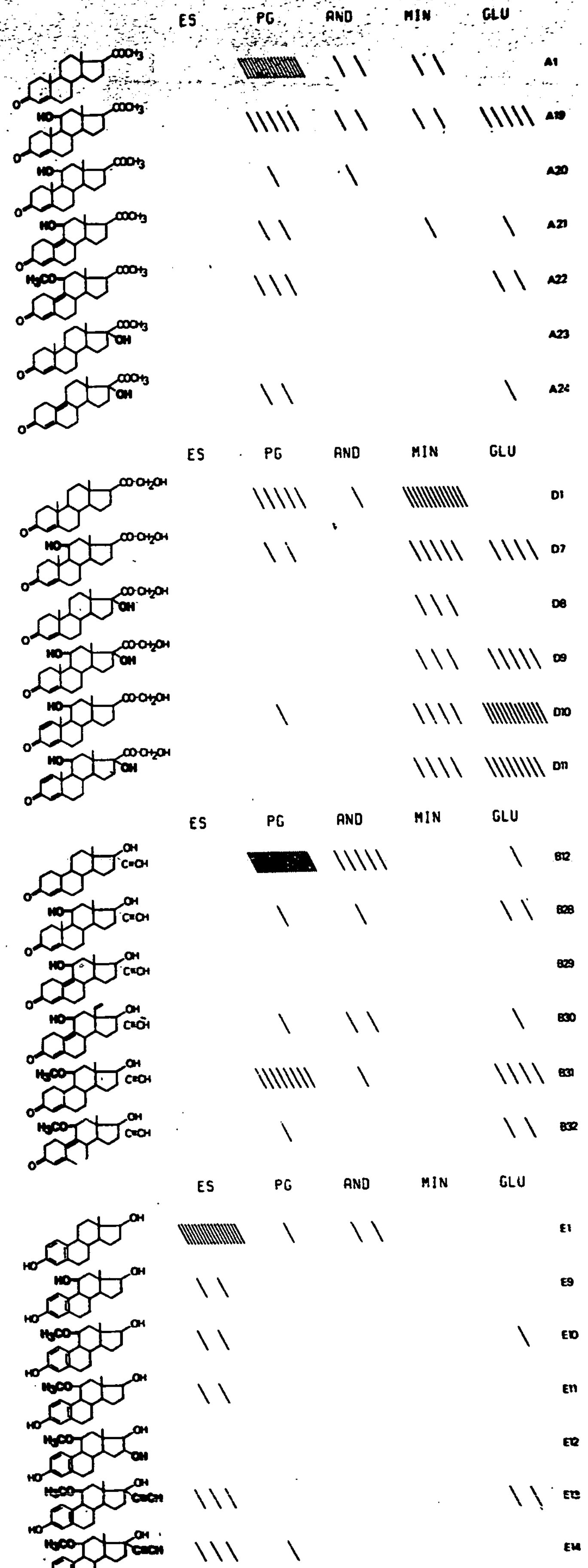


Chart 8. Effect of hydroxylation on the binding profiles of progesterone, testosterone, deoxycorticosterone, and estradiol to the estrogen (*ES*), progestin (*PG*), androgen (*AND*), mineralocorticoid (*MIN*) and glucocorticoid (*GLU*) receptors.

gestin and glucocorticoid binding (B31). In the estradiol series, methoxylation of the 11β- or α-hydroxy group has led to an interesting series of compounds (E10, E11, E13, and E14) with very similar binding profiles, but with very different activities. Although the RBA's of these compounds for the estrogen receptor after incubation for 2 hr at 0° are very similar, the kinetics of their interaction with the receptor is different. It would appear that the 11β-substituted compounds dissociate more slowly from the estrogen receptor than did estradiol and much more slowly than did the 11α-substituted compounds (9, 10), thus explaining the higher RBA's recorded for the 11β compounds and the lower RBA's recorded for the 11α compounds after long incubation times at high temperatures (Table 6). As a consequence, E10 and E13 are potent estrogens, and E11 and E14 exhibit antiestrogenic activity (8, 10, 29, 57).

The available 17α-hydroxy derivatives had little binding affinity for steroid hormone receptors, *e.g.*, A23, A24, and D8. 11,17-Dihydroxy-substituted corticosteroids (D9 and D11) had very similar profiles to the 11-mono-substituted compounds (D7 and D10).

Conformation Studies

Of the molecules listed in Charts 5 to 8, one of the most aspecific is B17, which binds fairly markedly to 3 hormone receptors (the progestin, androgen, and glucocorticoid receptors). One of the possible reasons for this versatile binding may reside in the flexibility of this steroid, as revealed by X-ray crystallography (J. P. Mornon, personal communication). Chart 9 illustrates the different conformations identified in crystals which, on examination, can be shown to possess a certain degree of overlap with published conformations for testosterone and progesterone (43). This overlap could afford a partial explanation for the androgen and progestin binding components of this molecule, a point which is at present under further study.

Discussion

The above results on 81 selected molecules suggest that a systematic study of the effect of various substituents on the receptor binding profile of natural steroid hormones could lead to the conception of new highly specific ligands. When, furthermore, these ligands do not bind to specific plasma proteins, are not degraded on *in vitro* incubation with cytosol, can be labeled to high specific radioactivity, and have low nonspecific binding, then these new molecules may prove invaluable in the detection and assay of hormone receptors in human tissues. However, the aim will probably not be easily achieved, since these and other

Table 6

RBA's of estradiol derivatives under different incubation conditions for the cytoplasmic estrogen receptor in mouse uterus

	2 hr at 0°	5 hr at 25°
Estradiol (E1)	100	100
E10	5.9 (4)[a]	31
E11	4.3 (2)	0.3 (1)
E13	12 (9)	122 (7)
E14	13 (15)	4 (7)

[a] Numbers in parentheses, number of determinations.

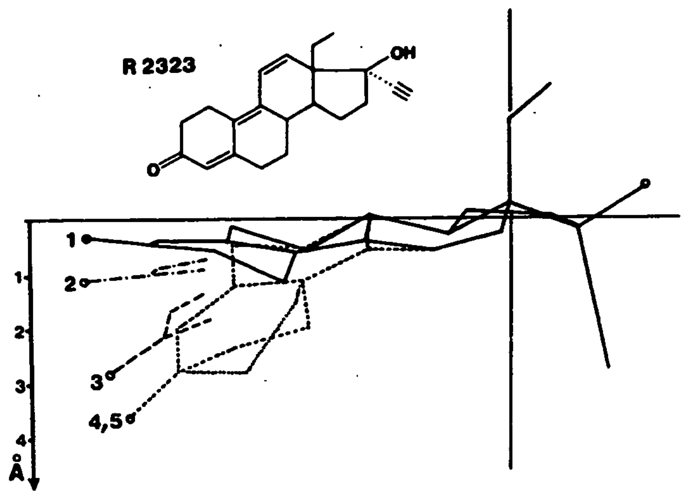


Chart 9. Projections obtained on X-ray diffraction analysis of B17. The rectangular coordinates system used to represent the molecule has its origin at the center of gravity of ring D (43).

results (60) suggest that there is a high degree of similarity among the receptors which bind 3-keto-Δ4 steroids and, consequently, that a single substituent can often interfere with binding to several receptors.

Available radioligands, in particular those used to label the receptors in the above studies, have already constituted a decisive improvement over labeled natural hormones, although none, except for the estrogen R 2858, fulfil all the criteria for an ideal ligand. R 2858 does not bind with high affinity to plasma proteins, interacts firmly with the estrogen receptor, is not degraded *in vitro* on incubation at 25°, and has low nonspecific binding. On the other hand, an improvement on the radioligand used to label the androgen receptor (R 1881) could be obtained by increasing receptor specificity, and on the radioligand used to label the progestin receptor (R 5020) an improvement could be obtained by decreasing nonspecific binding. Whether these improvements are worthwhile in the light of present knowledge and in view of the number of control studies required to develop a new radioligand remains a moot point.

In the absence of ideal tags, methods have to be found to palliate their disadvantages. Two will be suggested here. First, addition of an excess of a competitor which binds to the secondary receptors, *e.g.*, addition of triamcinolone acetonide (to labeled R 1881), which binds to the progestin, mineralo- and glucocorticoid binding components (W. M. McGuire, personal communication; J. Asselin, personal communication) and thus eliminates their interference. This procedure is analogous to the addition of excess specific plasma binder, *e.g.*, addition of cortisol to eliminate progesterone binding to CBG. Secondly, choosing the experimental conditions which compensate lack of specificity by favoring binding to one receptor rather than to another (4). This method is at present under further study.

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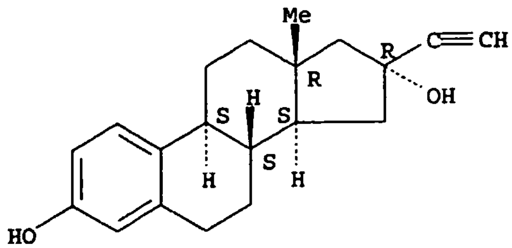
References

1. Asselin, J., Labrie, F., Gourdeau, Y., Bonne, C., and Raynaud, J. P. Binding of (3H) Methyltrienolone (R 1881) in Rat Prostate and Human Benign Prostatic Hypertrophy (BPH). Steroids, *28:* 449–459, 1976.
2. Azadian-Boulanger, G., and Bertin, D. Synthèse et Activité Utérotrophique des 11β-methoxy Estradiol, 11β-Methoxy Estriol, et 11β-Methoxy 17α-Ethynyl Estradiol. Chimie Thérapeutique, *8:* 451–454, 1973.
3. Azadian-Boulanger, G., Bucourt, R., Nedelec, L., and Nomine, G. Stéroides Trieniques Androgènes et Anabolisants. European J. Med. Chem., *10:* 353–359, 1975.
4. Boesel, R. W., Klipper, R. W., and Shain, S. A. Identification of Limited Capacity Androgen Binding Components in Nuclear and Cytoplasmic Fractions of Canine Prostate. Endocrine Res. Commun., *4:* 71–84, 1977.
5. Bonne, C., and Raynaud, J. P. Activité Anti-androgène du R 2956 (17-β-Hydroxy-2,2,17α-trimethyl-estra-4,9,11-trien-3-one). II. Mécanisme d'Action. J. Pharmacol. *5:* 521–532, 1974.
6. Bonne, C., and Raynaud, J. P. Methyltrienolone, a Specific Ligand for Cellular Androgen Receptors. Steroids, *26:* 227–232, 1975.
7. Bonne, C., and Raynaud, J. P. Assay of Androgen Binding Sites by Exchange with Methyltrienolone (R 1881). Steroids, *27:* 497–507, 1976.
8. Bouton, M. M., Fortin, M., Tournemine, C., and Philibert, D. Relations between Binding Kinetics and Biological Response. *In:* Abstracts, 7th International Congress of Pharmacology, Paris, p. 265, 1978.
9. Bouton, M. M., and Raynaud, J. P. The Relevance of Kinetic Parameters in the Determination of Specific Binding to the Estrogen Receptor. J. Steroid Biochem., *9:* 9–15, 1977.
10. Bouton, M. M., and Raynaud, J. P. Impaired Nuclear Translocation and Regulation: A Possible Explanation of Anti-Estrogenic Activity. *In:* A. Vermeulen, A. Klopper, F. Sciarra, P. Jungblut, and L. Lerner (eds.), Research on Steroids, Vol. VII, pp. 127–138. Amsterdam: Elsevier (North Holland Biomedical Press), 1977.
11. Bucourt, R., Tessier, J., and Nomine, G. Extension de la Synthèse Totale Stéroide à la nor-19-progesterone. Bull. Soc. Chim. France, 8–9, 1923–1925, 1963.
12. Chan, D. W., and Slaunwhite, W. R., Jr. The Binding of a Synthetic Progestin, R 5020, to Transcortin and Serum Albumin. J. Clin. Endocrinol. Metab., *44:* 983–985, 1977.
13. Cowan, R. A., Cowan, S. K., and Grant, J. K. Binding of Methyltrienolone (R 1881) to a Progesterone Receptor-like Component of Human Prostatic Cytosol. J.Endocrinol., *74:* 281–289, 1977.
14. Desombre, E. R., and Jensen, E. V. Steroid Receptors in Breast Neoplasia. *In:* W. E. Criss, T. Ono, and J. R. Sabine (eds.), Control Mechanisms in Cancer, pp. 67–82. New York: Raven Press, 1976.
15. Disorbo, D., Rosen, F., McPartland, R. P., and Milholland, R. J. Glucocorticoid Activity of Various Progesterone Analogs: Correlation between Specific Binding in Thymus and Liver and Biologic Activity. Ann. N. Y. Acad. Sci., *286:* 355–368, 1977.
16. Dube, J. Y., Chapdelaine, P., Tremblay, R. R., Bonne, C., and Raynaud, J. P. Comparative Binding Specificity of Methyltrienolone in Human and Rat Prostate. Hormone Res., *7:* 341–347, 1976.
17. Engelsman, E., Korsten, C. B., Persijn, J. P., and Cleton, F. J. Oestrogen and Androgen Receptors in Human Breast Cancer. Brit. J. Cancer, *30:* 177, 1974.
18. Fazekas, A. G., and McFarlane, J. K. Macromolecular Binding of Glucocorticoids in Human Mammary Carcinoma. Cancer Res., *37:* 640–645, 1977.
19. Feil, P. D., Glasser, S. R., Toft, D. O., and O'Malley, B. W. Progesterone Binding in the Mouse and Rat Uterus. Endocrinology, *91:* 738–746, 1972.
20. Fuller, P. J., and Funder, J. W. Mineralocorticoid and Glucocorticoid Receptors in Human Kidney. Kidney Intern., *10:* 154–157, 1976.
21. Giannopoulos, G. A. A Specific Glucorticoid Binding Macromolecule of Rabbit Uterine Cytosol. Biochem. Biophys. Res. Commun., *54:* 600–606, 1973.
22. Grover, P. K., and Odell, W. D. Correlation of *in Vivo* and *in Vitro* Activities of Some Naturally Occurring Androgens Using a Radioreceptor Assay for 5α-Dihydrotestosterone with Rat Prostate Cytosol Receptor Protein. J. Steroid Biochem., *6:* 1373–1379, 1975.
23. Gustafsson, J. A., Ekman, P., Pousette, Å., Snochowski, M., and Hogberg, B. Demonstration of a Progestin Receptor in Human Benign Prostatic Hyperplasia and Prostatic Carcinoma. J. Clin. Endocrinol. Metab., *45:* 920–930, 1977.
24. Hähnel, R., Twaddle, E., and Ratajczak, T. The Specificity of the Estrogen Receptor of Human Uterus. J. Steroid Biochem., *4:* 21–31, 1973.
25. Horwitz, K. B., and McGuire, W. L. Specific Progesterone Receptors in Human Breast Cancer. Steroids, *25:* 497–505, 1975.
26. Hospital, M., Busetta, B., Courseille, C., and Precigoux, G. X-ray Conformation of Some Estrogens and Their Binding to Uterine Receptors. J. Steroid Biochem., *6:* 221–225, 1975.
27. Karr, J. P., Kirdani, R. Y., Murphy, G. P., and Sandberg, A. A. Sex Hormone Binding Globulin and Transcortin in Human and Baboon Males. Arch. Androl., *1:* 123–129, 1978.
28. Katzenellenbogen, J. A., Johnson, H. J., Jr., and Myers, H. N. Photoaffinity Labels for Estrogen Binding Proteins of Rat Uterus. Biochemistry, *12:* 4085–4092, 1973.
29. Kelly, P. A., Asselin, J., Caron, M. G., Raynaud, J. P., and Labrie, F. High Inhibitory Activity of a New Antiestrogen, RU 16117 (11α-Methoxy Ethinyl Estradiol), on the Development of Dimethylbenz(a)anthracene-induced Mammary Tumors. Cancer Res., *37:* 76–81, 1977.
30. King, R. J. B., and Mainwaring, W. I. P. Steroid-Cell Interactions, London: Butterworth & Co., 1974.
31. Kontula, K., Jänne, O., Luukkainen, T., and Vihko, R. Progesterone Binding Protein in Human Myometrium. Ligand Specificity and Some Physicochemical Characteristics. Biochim. Biophys. Acta, *328:* 145–153, 1973.
32. Kontula, K., Jänne, O., Vihko, R., De Jager, E., De Visser, J., and Zeelen, F. Progesterone-binding Proteins: *In Vitro* Binding and Biological Activity of Different Steroidal Ligands. Acta Endocrinol., *78:* 574–592, 1975.
33. Korenman, S. G. Comparative Binding Affinity of Estrogens and Its Relation to Estrogenic Potency. Steroids, *13:* 163–177, 1969.
34. Krieg, M., Bartsch, W., Herzer, S., Becker, H., and Voigt, K. D. Quantification of Androgen Binding, Androgen Tissue Levels, and Sex Hormone-binding Globulin in Prostate, Muscle and Plasma of Patients with Benign Prostatic Hypertrophy. Acta Endocrinol., *86:* 200–215, 1977.
35. Leavitt, W. W., Toft, D. O., Strott, C. A., and O'Malley, B. W. A Specific Progesterone Receptor in the Hamster Uterus: Physiologic Properties and Regulation during the Estrous Cycle. Endocrinology, *94:* 1041–1053, 1974.
36. Liao, S., Liang, T., Fang, S., Castaneda, E., and Shao, T. C. Steroid Structure and Androgenic Activity. Specificities Involved in the Receptor Binding and Nuclear Retention of Various Androgens. J. Biol. Chem., *248:* 6154–6162, 1973.
37. Lippman, M., and Huff, K. A. A Demonstration of Androgen and Estrogen Receptors in a Human Breast Cancer Using a New Protamine Sulfate Assay. Cancer, *38:* 868–874, 1976.
38. Maass, H., Engel, B., Trams, G., Nowakowski, H., and Stolzenbach, G. Steroid Hormone Receptors in Human Breast Cancer and the Clinical Significance. J. Steroid Biochem., *6:* 743–749, 1975.
39. Martin, J. E., Philpott, J., Chatkoff, M., Young, R., and Pauerstein, C. J. Effect of Progesterone on Estrogen Receptors in the Rabbit Uterus. Am. J. Obstet. Gynecol., *121:* 180–186, 1975.
40. McGuire, J. L., Bariso, C. D., and Shroff, A. P. Interaction between Steroids and a Uterine Progestogen Specific Binding Macromolecule. Biochemistry, *13:* 319–322, 1974.
41. McGuire, W. L., Horwitz, K. B., Zava, D. T., Garola, R. E., and Chamness, G. C. Hormones Breast Cancer—Update 1978. Metabolism, *27:* 487–501, 1978.
42. McGuire, W. L., Raynaud, J. P., and Baulieu, E. E. (eds.). Progesterone Receptors in Normal and Neoplastic Tissues. New York: Raven Press, 1977.
43. Mornon, J. P., Delettre, J., Lepicard, G., Bally, R., Surcouf, E., and Bondot, P. Interactions of Hormonal Steroids: Progestogens. J. Steroid Biochem., *8:* 51–62, 1977.
44. Nedelec, L., Gasc, J. C., and Bardoneschi, R. A Potent Antiandrogenic Steroid, the 17β-OH-2,2,17α-trimethyl-3-oxo-estra-4,9,11-triene, Obtained by a New Methylation Procedure. *In:* Abstracts, Third International Congress on Hormonal Steroids, Hamburg, West Germany, 1970, ICS 210, No. 167. Amsterdam: Excerpta Medica, 1970.
45. Nunez, E. A., Benassayag, C., Savu, L., Vallette, G., and Jayle, M. F. Serum Binding of Some Steroid Hormones during Development in Different Animal Species. Discussion on the Biological Significance of This Binding. Ann. Biol. Animale Biochim. Biophys., *16:* 491–501, 1976.
46. Ochi, H., Hayashi, T., Nakao, K., Yayoi, E., Kawahara, T., and Matsumoto, K. Estrogen, Progesterone and Androgen Receptors in Breast Cancer in Japanese. J. Natl. Cancer Inst., *60:* 291–293, 1978.
47. Philibert, D., Ojasoo, T., and Raynaud, J. P. Properties of the Cytoplasmic Progestin Binding Protein in the Rabbit Uterus. Endocrinology, *101:* 1850–1861, 1977.
48. Philibert, D., and Raynaud, J. P. Progesterone Binding in the Immature Mouse and Rat Uterus. Steroids, *22:* 89–98, 1973.
49. Philibert, D., and Raynaud, J. P. Cytoplasmic Progestin Receptors in Mouse Uterus. *In:* W. L. McGuire, J. P. Raynaud, and E. E. Baulieu, (eds.), Progesterone Receptors in Normal and Neoplastic Tissues, pp. 227–243. New York: Raven Press, 1977.
50. Philbert, D., and Raynaud, J. P. Properties of the Glucocorticoid Receptor in Rat Tissues and in HTC cells. *In:* Abstracts, Fifth International Congress on Hormonal Steroids, New Delhi, India, October 1978.
51. Pollow, K., Lubbert, H., Boquoi, E., Kreuzer, G., and Pollow, B. Characterization and Comparison of Receptors for 17β-Estradiol and Progesterone in Human Proliferative Endometrium and Endometrial Carcinoma. Endocrinology, *96:* 319–328, 1975.
52. Poortman, J., Prenen, J. A. C., Schwartz, F., and Thijssen, J. H. H. Interaction of Δ5-Androstene-3β,17β-diol with Estradiol and Dihydrotestosterone Receptors in Human Myometrial and Mammary Cancer Tissue. J. Clin. Endocrinol. Metab., *40:* 373–379, 1975.

53. Raynaud, J. P. Influence of Rat Estradiol Binding Plasma Protein (EBP) on Uterotrophic Activity. Steroids, *21:* 249–258, 1973.
54. Raynaud, J. P. R 5020, a Tag for the Progestin Receptor. *In:* W. L. McGuire, J. P. Raynaud, and E. E. Baulieu (eds.), Progesterone Receptors in Normal and Neoplastic Tissues, pp. 9–21. New York: Raven Press, 1977.
55. Raynaud, J. P. A Strategy for the Design of Potent Hormones: *In:* J. Mathieu (ed.), Medicinal Chemistry, Vol. V, pp. 451–456. Amsterdam: Elsevier Scientific Publishing Co., 1977.
56. Raynaud, J. P. Une Stratégie de Recherche pour les Hormones de Synthèse: leurs Interactions avec les Récepteurs Hormonaux. *In:* J. Cheymol, J. R. Boissier, and P. Lechat (eds.), Actualités Pharmacologiques, No. 29, pp. 49–64. Paris: Masson et Cie, 1977.
57. Raynaud, J. P., Bouton, M. M., Gallet-Bourquin, D., Philibert, D., Tournemine, C., and Azadian-Boulanger, G. Comparative Study of Estrogen Action. Mol. Pharmacol., *9:* 520–533, 1973.
58. Raynaud, J. P., Martin, P., Bouton, M. M., and Ojasoo, T. Moxestrol, a Tag for the Estrogen Receptor. Cancer Res., in press, *38:* 0000–0000, 1978.
59. Raynaud, J. P., Mercier-Bodard, C., and Baulieu, E. E. Rat Estradiol Binding Plasma Protein (EBP). Steroids, *18:* 767–788, 1971.
60. Raynaud, J. P., Ojasoo, T., Bouton, M. M., and Philibert, D. Receptor Binding as a Tool in the Development of New Bioactive Steroids. *In:* E. J. Ariens (ed.), Drug Design, Vol. VIII, pp. 170–214, New York: Academic Press, Inc., 1978.
61. Raynaud, J. P., Ojasoo, T., Delarue, J. C., Magdelenat, H., Martin, P., and Philibert, D. Estrogen and Progestin Receptors in Human Breast Cancer. *In:* W. L. McGuire, J. P. Raynaud, and E. E. Baulieu, (eds.), pp. 171–191. Progesterone Receptors in Normal and Neoplastic Tissues, New York: Raven Press, 1977.
62. Raynaud, J. P., Ojasoo, T., and Vache, V. Unusual Steroids in Measuring Steroid Receptors. *In:* E. B. Thompson and M. E. Lippman (eds.), Steroid Receptors and the Management of Cancer, Cleveland: CRC Press, Inc., in press, 1978.
63. Roth, G. S. Age-related Changes in Specific Glucocorticoid Binding by Steroid-responsive Tissues of Rats. Endocrinology, *94:* 82–90, 1973.
64. Shain, S. A., and Boesel, R. W. Saturation Analysis of the Binding of Androgens, Antiandrogens and Estrogens by the Cytoplasmic High Affinity Androgen Receptor of the Rat Ventral Prostate. J. Steroid Biochem., *6:* 43–50, 1975.
65. Shutt, D. A., and Cox, R. I. Steroid and Phyto-oestrogen Binding to Sheep Uterine Receptors 'in vitro'. J. Endocrinol., *52:* 299–310, 1972.
66. Smith, H. E., Smith, R. G., Toft, D. O., Neergaard, J. R., Burrows, E. P. and O'Malley, B. W. Binding of Steroids to Progesterone Receptor Proteins in Chick Oviduct and Human Uterus. J. Biol. Chem., *249:* 5924–5932, 1974.
67. Snochowski, M., Pousette, Å., Ekman, P., Bression, D., Andersson, L., Hogberg, B., and Gustafsson, J. Å. Characterization and Measurement of the Androgen Receptor in Human Benign Prostatic Hyperplasia and Prostatic Carcinoma. J. Clin. Endocrinol. Metab., *45:* 920–930, 1977.
68. Terenius, L. Estrogen and Progestogen Binders in Human and Rat Mammary Carcinoma. European J. Cancer, *9:* 291–294, 1973.
69. Terenius, L. Studies on Progestogen Receptors in Breast Cancer with a Synthetic Progestogen, DU-41165. Advan. Tumour Prevent. Detection Characteristics, *3:* 321–323, 1976.
70. Teulings, F. A. G., and Van Gilse, H. A. Demonstration of Glucocorticoid Receptors in Human Mammary Carcinomas. Hormone Res., *8:* 107–116, 1977.
71. Trams, G., and Maass, H. Specific Binding of Estradiol and Dihydrotestosterone in Human Mammary Cancers. Cancer Res., *37:* 258–261, 1977.
72. Van Beurden-Lamers, W. M. O., Brinkmann, A. O., Mulder, E., and Van der Molen, H. J. High-affinity Binding of Oestradiol-17β by Cytosols from Testis Interstitial Tissue, Pituitary, Adrenal, Liver and Accessory Sex Glands of the Male Rat. Biochem. J., *140:* 495–502, 1974.
73. Velluz, L., Nomine, G., Bucourt, R., Pierdet, A., and Tessier, J. L'extension de la Synthèse totale dans le Groupe des Nortestostérones. Compt. Rend Acad. Sci., *252:* 3903–3905, 1961.
74. Velluz, L., Nomine, G., Mathieu, J., Toromanoff, E., Bertin, D., Tessier, J., and Pierdet, A. Sur l'accès Stéréospecifique par Synthèse Totale, à la Série 19-nor-stéroïde. La 19-nor-testostérone de synthèse. Compt. Rend. Acad. Sci., *250:* 1084–1085, 1960.
75. Velluz, L., Valls, J., and Nomine, G. Fortschritte in der Totalsynthese von Steroiden. Angew. Chem. Intern. Ed. Engl., *77:* 185–205, 1965.
76. Wagner, R. K., and Jungblut, P. W. Differentiation between Steroid Hormone Receptors CBG and SHBG in Human Target Organ Extracts by a Single-step Assay. Molecular Cell Endocrinol., *4:* 13–24, 1976.
77. Wagner, R. K., Schulze, K. H., and Jungblut, P. W. Estrogen and Androgen Receptor in Human Prostate and Prostatic Tumor Tissue. Acta Endocrinol. *193* (Suppl.): 52, 1975.
78. Westphal, U. Steroid-Protein Interactions. Berlin: Springer-Verlag, 1971.



AB Anal. of potential endocrine disrupting chems. in wastewater effluent using continuous liquid-liquid extraction with derivatization and gas chromatog./mass spectrometry anal. is described.

L6 ANSWER 2 OF 3 CAPLUS
ACCESSION NUMBER: 1979:66957 CAPLUS
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CORPORATE SOURCE: Cent. Rech., Roussel-UCLAF, Romainville, Fr.
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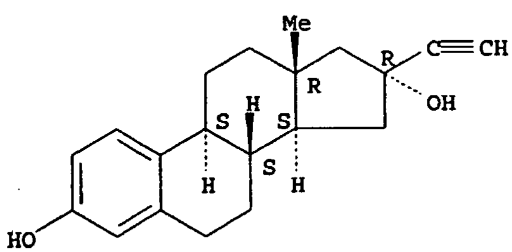
IT **24989-47-7**
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(receptor binding of, structure in relation to)
RN 24989-47-7 CAPLUS
CN Estra-1,3,5(10)-triene-3,16-diol, 16-ethynyl-, (16α)- (9CI) (CA INDEX NAME)

Absolute stereochemistry.



AB A simple in vitro system was used to define the mol. requirements for a highly specific interaction between a steroid and the receptor corresponding to a single class of hormone. Homogenates or crude 105,000 g supernatant were prepared from the target organs considered as end points in routine biol. potency tests. Available radioligands not bound by plasma proteins (tags) were used to single out the receptors. For each receptor singled out in the target organ cytoplasm, the ability of >700 mols. to decrease bound radioactivity was compared to that of the natural hormone (relative. binding affinity) with the use of a dextran-coated charcoal technique to sep. bound from unbound steroid. On the basis of the results of 81 mols., the effect of various substituents on the

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Iodoestrogens, Syntheses, and Interaction with Uterine Receptors*

(Received for publication, September 5, 1978)

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The rationale for undertaking the present study was to evaluate the utility of iodoestradiol analogs made highly radioactive with iodine isotopes in (*a*) the noninvasive differentiation of estrogen-dependent from estrogen-independent breast tumors, (*b*) spread of metastases containing estrogen receptors, and (*c*) potential application in therapeutic irradiation of target tissues. In the present paper, the model syntheses of a number of nonradioactive ^{127}I-estrogen analogs are described. The analogs were tested for their ability to displace (compete with) [^{3}H]estradiol from receptor sites. The most active compounds, 16β-iodoestra-1,3,5(10)-triene-3,17β-diol (*17*) and 6-iodoestra-1,3,5(10),6-tetraene-3,17β-diol (*10b*), showed a relative binding affinity of 0.57 and 0.49, respectively.

It is recognized that estrogen receptors can be found in about 50 to 70% of tumors from breast cancer patients. In about 50% of these patients hormonal manipulative therapy will result in tumor remission while only rare tumors, if estrogen receptor negative will remit with similar hormonal therapy (1-3). Thus, it has been suggested that the tumors of all breast cancer patients be assayed for the presence of estrogen receptors. Unfortunately, at present assaying for estrogen receptors can be carried out only *in vitro*, and the tissues must be first excised in part, or *in toto* (1, 3). There is an obvious dire need for a rapid noninvasive *in vivo* method of defining the estrogen receptor status of the neoplasia, which would facilitate the designing of a therapeutic approach to the patient. In this paper we describe our approach to the problem and report some preliminary results of our *in vitro* investigations.

Our plan was to prepare highly radioactive estrogen analogs capable of competing with estradiol for receptor sites and administer these to patients. We rationalized that if estrogen receptors were to be present in the tumor (and metastases) some of the administered labeled analogs would be retained at the receptor sites of the patient. In practice, the receptor-positive and receptor-negative tumors (and metastases) could, therefore, be differentiated by measuring externally the accumulation (concentration) of radioactivity in the tumor and metastases relative to the normal surroundings tissues. This, of necessity, requires the use of one of the more energetic radioisotopes for steroid labeling.

In a search for nuclides suitable for external *in vivo* scanning, we turned our attention toward several strong γ emitters. We first focused on the possible use of certain radioactive isotopes of iodine because they emit hard γ-radiation, can be obtained with a high specific activity (about 2200 Ci/mmol), and the chemistry of iodination of steroids has been previously developed. Consequently, we undertook to prepare model ^{127}I-estrogen analogs and evaluate their ability to compete with [^{3}H]estradiol for receptor sites *in vitro*. The results of these studies are described herein.

EXPERIMENTAL PROCEDURES AND RESULTS[1]

DISCUSSION

Although tritium and carbon 14 remain the usual radioactive isotopes for labeling steroids, for the past several years attempts have been made to use other radioactive isotopes for this purpose. These isotopes include 18-fluorine (4) and 75-selenium (5), but the commonest have been the radioactive isotopes of iodine (6-13). So far the results of these endeavors have not been very successful in part due to nonspecific labeling techniques, to loss of biologic activity following iodination, or to nonspecific binding of the iodinated molecule (8, 13). We have attempted to overcome these problems through specific iodination at sites which will not destroy the specific receptor binding and the biological activity of the iodinated estrogens. The results of our investigations are summarized in Tables I and II and Figs. 1 to 3.[1]

Despite the bulk of the iodine atom which replaces a hydrogen H atom in the steroid structure, the binding of the subsequent iodinated estrogen is relatively high for several of these compounds. In these compounds, the iodine atom can be placed in either the 4, 6, 7, or 16 position, and the ability to bind to the receptor is retained. Katzenellenbogen (14) has previously noted that substituents on these groups are well tolerated so far as receptor binding is concerned. Insertion of an iodine atom in the 2 or the 17 position, however, will markedly hinder the binding affinity of the iodinated estrogen to its receptor. This is proabably due to the site involved and not the iodine atom *per se*, since the 2-hydroxylated estrogens also bind very poorly to the uterine receptor (15). We noted in *in vivo* studies that the iodinated steroid could enter the circulation, be carried to the target tissue, enter the target cells, and compete directly with [^{3}H]estradiol for receptors in the cells. Following the *in vivo* administration of [^{3}H]estradiol with and without nonradioactive compounds, it can be calculated that about 0.8 nmol of the iodinated compound is present at specific receptor sites/100 mg of uterus.

Since we used ^{127}I for these experiments we could not assess the extent of nonspecific binding to other tissues which has been noted by others (8, 13). However, in one experiment in which Compound 10b was labeled with ^{125}I, *in vivo* studies

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[1] Portions of this paper (including the syntheses and "References") are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, Md. 20014. Request Document No. 78M-1589, cite author(s), and include a check or money order for $2.85 per set of photocopies.

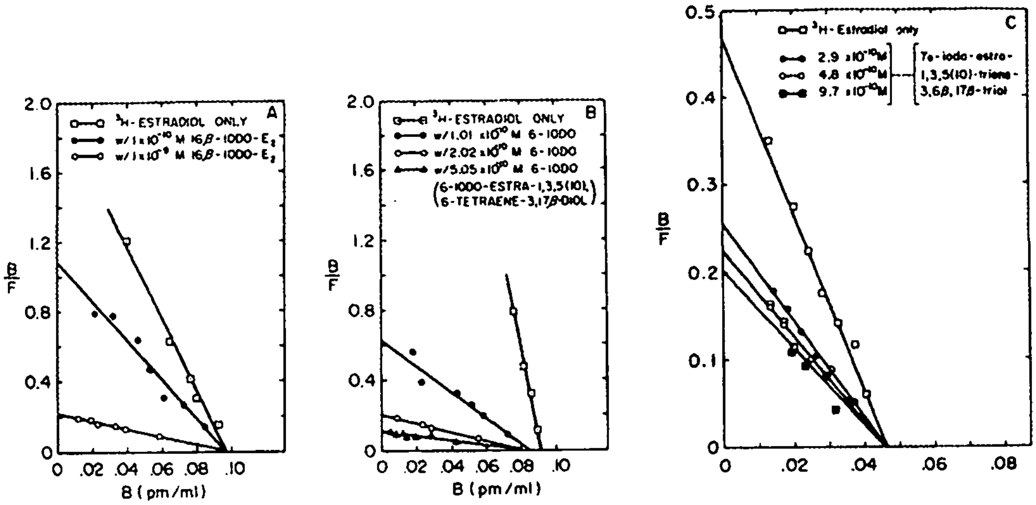

FIG. 1. Scatchard plots (23) of bound/free (*B/F*) *versus* (*B*) bound for incremental amounts of [^{3}H]estradiol incubated in the absence, □——□, and in the presence of: *A*, 16β-iodoestradiol (*17*), 1 × 10^{-10} M, ●——●, or 1 × 10^{-9} M, ○——○; *B*, 6-iodoestra-1,3,5(10),6-tetraene-3,17β-diol (*10b*), 1.01 × 10^{-10} M, ●——●, 2.02 × 10^{-10} M, ○——○, or 5.05 × 10^{-10} M, ▲——▲; *C*, 7α-iodoestra-1,3,5(10)-triene-3,6β,17β-triol (*11b*), 2.9 × 10^{-10} M, ●——●, 4.8 × 10^{-10} M, ○——○, or 9.7 × 10^{-10} M, ■——■.

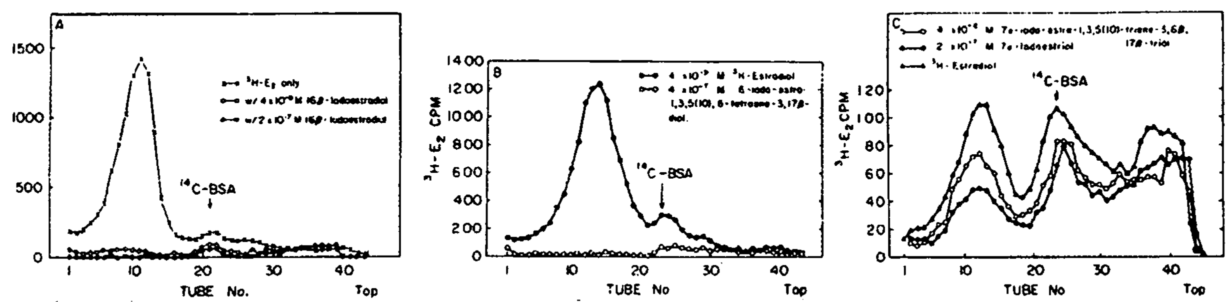

FIG. 2. Results of sucrose gradients of uterine cytosol incubated with [^{3}H]estradiol in the absence or presence of iodinated estrogens. *A*, [^{3}H]estradiol, ×——×, [^{3}H]estradiol plus 16-iodoestradiol (*17*) (4 × 10^{-8} M, ○——○; 2 × 10^{-7} M, ●——●). *B*, [^{3}H]estradiol, ●——●; [^{3}H]estradiol plus 6-iodoestra-1,3,5(10),6-tetraene-3,17β-diol (*10b*), 4 × 10^{-9} M, ○——○. *C*, [^{3}H]estradiol, ▲——▲; [^{3}H]estradiol plus 7α-iodoestra-1,3,5(10)-triene-3,6β,17β-triol (*11b*) (○——○, 4 × 10^{-8} M; ●——●, 2 × 10^{-7} M). *BSA*, bovine serum albumin.

TABLE I

Relative binding affinities (weak displacers)

Compound	Relative binding affinity[a]
Estra-1,3,5(10)-trien-3,17β-diol (*1*)	1.00
17α-Iodoestra-1,3,5(10)-triene-3,16β-diol (*19a*)	0.01
17α-Iodoestra-1,3,5(10)-triene-3-ol (*2a*)	0.01
17α-Iodoestra-1,3,5(10),6-tetraene-3-ol (*9c*)	<0.001
2,4,17-Triiodoestra-1,3,5(10),16-tetraene-3-ol (*6a*)	<0.001
17-Iodoestra-1,3,5(10)-16-tetraene-3-ol (*6b*)	<0.001
2,6-Diiodoestra-1,3,5(10),6-tetraene-3,17β-diol (*10a*)	<0.001
2-Iodoestra-1,3,5(10)-trien-3,17β-diol (*23*)	<0.001
2,4,17α-Triiodoestra-1,3,5(10)-triene-3,16β-diol (*19b*)	<0.001
4,17α-Diiodoestra-1,3,5(10)-triene-3,16β-diol (*19c*)	<0.001

[a] Calculated at B/B_0 = 50% (44).

TABLE II

Relative binding affinities (strong displacers)

Compound	Relative binding affinity[a]
Estradiol (*1a*)	1.00
16β-Iodoestra-1,3,5(10)-triene-3,17β-diol (*17*)	0.57
6-Iodoestra-1,3,5(10),6-tetraene-3,17β-diol (*10b*)	0.49
16α-Iodo-3-hydroxyestra-1,3,5(10)-trien-17-one (*22b*)	0.29
17α-Iodomethyl-estra-1,3,5(10)-trien-3,17β-diol (*21*)	0.09
3-Acetoxy-16α-iodoestra-1,3,5(10)-trien-17-one (*22a*)	0.07
4-Iodo-3-hydroxyestra-1,3,5(10),9(11)-tetraene-17-one (*14*)	0.05
7α-Iodo-3,17β-diacetoxyestra-1,3,5(10)-triene-6-one (*12*)	0.02
7α-Iodoestra-1,3,5(10)-triene-3,6β,17β-triol (*11b*)	0.02
7α-Iodo-3,6β-17β-trihydroxyestra-1,3,5(10)-triene-3,17-diacetate (*11a*)	0.01

[a] Calculated at B/B_0 = 50% (44).

showed that the nonspecific binding was similar to that found for [^{3}H]estradiol.[2] Although the results of the *in vivo* experiments are encouraging, further work is necessary before it can be shown that the described iodinated estrogen(s) will bind only to specific target sites and the radioactivity can be detected exogenously.

The possibility that some deiodination occurred under *in vitro* as well as *in vivo* conditions cannot be excluded. However, our results cannot be explained simply on the loss of iodine during the incubation procedures.

There was a rough correlation between the K_i values calculated from the competition experiments and the respective RBA values of the compounds tested. The results of the competition experiments are compatible with the conclusion

[2] See footnotes in the miniprint.

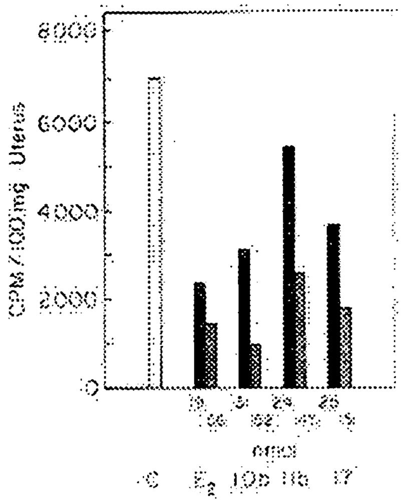

FIG. 3. Counts per min of [^{3}H]estradiol retained/100 mg of rat uterus 2 h following injection of [^{3}H]estradiol (2 μCi) in the absence, C, or presence of estradiol (E_2), 19 or 56 nmol, 6-iodoestra-1,3,5(10),6-tetraene-3,17β-diol (*10b*); 31 or 152 nmol, 7α-iodoestra-1,3,5(10)-triene-3,6β,17β-triol (*11b*), 24 or 145 nmol, or 16β-iodoestradiol (*17*) 25 or 151 nmol.

that the inhibition of estradiol binding by the iodinated estrogens is competitive in nature. They do not rule out the possibility, however, that covalent binding occurs between the iodine atom and certain amino acids of the receptor (14, 16). This type of binding has been reported to occur between the enzyme receptors and mercuriestrogens (17), iodinated corticoids (18), and steroids labeled with a bromine atom (14, 19).

Korenman (20), in studying the binding of estrogen compounds to rabbit uterine cytosol, noted that the acetates of estradiol and estrone bound at least as well as the parent compounds. We also found that the introduction of acetate groups did not interfere significantly with binding. The introduction of the iodine group at the 16β position does not diminish binding to the same extent as 16α substitution, a finding compatible with the report of King and Mainwaring (21) on the difference in binding between estriol and epiestriol. Similarly, the acetates of 7-iodoestra-1,3,5(10)3,6,17β-triol bound as well, if not better than the parent compound. We have no ready explanation for this apparent increase in binding affinity.

REFERENCES

See p. 5905.

Iodoestrogens, Syntheses and Interaction

With Uterine Receptors

Thangavel Arunachalam, Christopher Longcope and

Eliahu Caspi

Synthetic Approaches to Iodo-estrogens

Treatment of estradiol (1a) with triphenylphosphite methiodide (22,2) (TPMI)[2] in DMF (25°; 18h) gave 17α-iodoestra-1,3,5(10)-trien-3-ol (2a) (50% yield). As expected, the reaction proceeded with inversion of configuration and this was confirmed by nmr spectrum which showed a doublet of doublets for the 17β-hydrogen (δ 4.43; J_1=7 Hz; J_2=2 Hz). The attempts to prepare the 17β-iodide (2b) by treatment of (2a) with NaI in boiling acetone (24,25), NaI in boiling ethyl-methyl ketone or diglyme or MgI_2 in ether at 0° (26) failed and resulted in the recovery of the starting material.

However, when (2a) was treated with NaI in boiling isopropanol, the 17-methyl-18-norestra-1,3,5(10);13(17)-tetraene-3-ol (3) was formed in ca. 90% yield. The rearranged product (3) was also obtained by heating a hexamethylphosphorous triamide solution (HMPT) of (2a) with NaI in the presence of 18-crown-6 ether.

In a further attempt to prepare the 17β-iodide (2b), 17α-estradiol (1c) was treated with TPMI in DMF. However, again the reaction did not proceed as desired and, instead, 17β-methyl-18-norestra-1,3,5(10), 13-tetraen-3-ol (4) was formed (no vinylic protons; δ 1.01 doublet J=7 Hz, C_{18}-H). The noted rearrangements are not surprising since it is known that the development of a C-17 carbonium ion may result in the migration of the C-13 methyl and formation of C-13(17) or C-13(14) olefinic products.

Under the circumstances, we explored the possibility of preparing (2b) via the reduction of (6b). Estrone (5a) was converted to the hydrazone (5b) and treated with excess of iodine in the presence of triethylamine (26). The major product of the reaction was the 2,4,17-tri-iodoestra-1,3,5(10), 16-tetraen-3-ol (6a), and the desired mono-iodide (6b) was obtained in ca. 5% yield. In contrast, when equimolar amounts of iodine and (5b) were used, the 17-mono-iodide (6b) was obtained as the sole product. Unfortunately, attempted hydrogenation of (6b) over 5% Pd on charcoal gave a complex mixture of products which was not investigated further. On prolonged hydrogenation, the removal of the 16-double bond was accomoanied by loss of iodine. It is worthy of note that hydrogenation of (6a) under the same conditions resulted in the recovery of most of the starting material.

We then turned our attention to the introduction of iodine in Ring B. Treatment of the 6α-hydroxy (8a) and 6β-hydroxy (8d) (28,29) with TPMI in DMF gave the 6-olefin (9a) in quantitative yields. Similarly, treatment of the 6α-tosylate (8c) or 6β-tosylate (8e) with NaI in acetone or isopropanol (?) resulted in the 6-olefin (9a). The diacetate (9a) was saponified and the resulting 6-dehydro-diol (9b) was treated with TPMI in DMF to yield the 17α-iodide (9c). Exposure of the triol (8b) to TPMI-DMF also gave the iodide (9c).

The 6-vinylic iodide (10b) was prepared in 50% yield by treating 3,17β-dihydroxyestra-1,3,5(10)-triene-6-hydrazone (7b) with a 0.5 molar equivalent of iodine in the presence of triethylamine (27). When an excess of iodine was used in the reaction, a (1:1) mixture of (10a) and (10b) was formed. Attempts to prepare the 6-iodides via hydrogenation of the C-6 double bond failed.

The reaction of the 6-en-diacetate (9a) with N-iodosuccinimide (NIS) and aqueous $HClO_4$ (30) gave the 6β-hydroxy-7α-iodo (11a) in ca. 95% yield. NMR δ 5.05 [d,(J=2 Hz) 6α-H]; 4.43 [t,(J=2 Hz) 7β-H]. The low field doublet indicates that the hydroxyl is indeed in the benzylic C-6 position. The C-6 location of the hydroxyl was confirmed by the oxidation of (11a) to the 6-ketone-7α-iodide (12) [IR 1675 cm^{-1}; NMR δ 4.78 [d,(J=2 Hz) 7β-H]. The 7α-iodo-estra-1,3,5(10)-trien-3,6β,17β-triol (11b) was prepared by exposing the six-dehydro-diol (9b) to NIS and aqueons $HClO_4$.

Treatment of 11α-hydroxyestrone (15)(31) with TPMI gave only the 9(11)-dehydroestrone (13) (32-34). Exposure of 9(11)-dehydroestrone (13) to HOI (NIS + aqueous $HClO_4$) resulted in the 4-iodo compound (14). This is in contrast to the reactions of (13) with HOBr, where it was reported that the 9α-hydroxy 11β-bromo product is formed (30).

Two types of Ring D iodohydrins were prepared. Treatment of estriol (16) with two equivalents of TPMI resulted in a mixture of products from which the iodohydrin (17) was isolated in 23% yield. In addition, the 16-olefin (18a) (30%) and the phosphonate ester analog (18b) (5%) were obtained. The iodohydrin (17) gave a positive Beilstein test; its mass spectrum had peaks at m/e 398 (M^+) and 270 [M-128;(M-HI)] (100%) and its nmr had a singlet (3H) at 0.96 (18-H) and a multiplet (2H) at 4.75 for the 16α- and 17β-hydrogen atoms. The product, on treatment with base, gave estrone as expected for a cis iodohydrin. Similarly, when (16) was treated with an equimolar amount of TPMI, the cis iodohydrin (17) was obtained in 30% yield. In contrast, with 4 equivalents of TPMI, the olefin (18a) was the main product (ca.70%), and only a small amount of (17) was formed.

Addition of hypoiodous acid to the olefin (18a) gave the trans-16β-hydroxy-17α-iodo product (19a) (75% yield). As expected, the nmr of (19a) exhibited a narrow doublet at 4.31 (J=1.5 Hz) for the 17β-hydrogen, and a multiplet at 5.16 for the 16α-hydrogen. Small amounts (ca. 5% each) of the tri-iodo (19b) and di-iodo (19c) were also isolated.

The second type of Ring D iodohydrins was obtained from the known oxirane (35) (20). The oxirane (20), on treatment with hydriodic acid (36), gave 17α-iodomethyl estradiol (21). The 16α-iodoestrone (22b) and its acetate (22a) and 2-iodoestradiol (23) were prepared by the methods described in the literature (37,38).

Experimental; Synthetic Procedures

Synthesis of Iodo-estrogens

Melting points were determined on a hot stage apparatus and are corrected. Infrared spectra (IR) were recorded on a Perkin-Elmer 237 spectrophotometer as KBr wafers. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian DA 60 spectrometer at 60 MHz or HR-100 at 100 MHz. Chemical shifts are quoted in δ values downfield from internal standard tetramethyl silane. Coupling constants are quoted in Hz. Mass spectra were obtained on a Nuclide 12-90G instrument, at 70 ev., by the direct insertion method. Thin layer chromatography (TLC) was carried out on plates coated with silica gel (Merck H.F. 254 + 366) and activated at 130° for 6 h. Column chromatography was carried out on silica gel (Merck). Estradiol and estrone were purchased from Steraloids, Inc.

17α-Iodoestra-1,3,5(10)-trien-3-ol (2a)

A solution of triphenylphosphite methiodide (TPMI) (22) (340 mg) and estradiol (1a) (175 mg) in dimethylformamide (DMF) (4 mL) was stirred at room temperature under nitrogen for 24 h. The reaction mixture was then added dropwise to a stirred mixture of ice and water (200 g). The obtained solid was separated by filtration and dissolved in ether. The ether solution was washed with 5% sodium thiosulphate and water, dried over anhydrous sodium sulfate and the solvent was removed at room temperature, under reduced pressure, to yield a gum (280 mg). The residue was fractionated by preparative TLC [silica gel hexane-ethyl acetate (3:1)] to give (2a) [105 mg; (50%)] mp 120-124° (dec). The compound turned pink within a few hours. NMR ($CDCl_3$) δ 0.85 (2,3H,C_{18}-H) 4.43 (1H, d of d, J_1=6 Hz, J_2=1.5 Hz, C_{17}-βH). Mass spectrum m/e 382 (M^+), 254 (M^+-HI).

Attempted Isomerization of 17α-iodoestra-1,3,5(10)-trien-3-ol (2a)

A mixture of the 17α-iodide (2a) (200 mg) and sodium iodide (400 mg) in acetone (10 mL) (or ethyl methyl ketone) was refluxed for 5 h under nitrogen (25). The solvent was concentrated to half of its original volume and ice cold water was added. The product was recovered with ether. The ether extract was washed with water, dried over Na_2SO_4 and the solvent removed to give a solid, which was identical to the starting material. Attempts to isomerize the 17α-iodide (2a) by treatment with magnesium iodide (6b) in ether at 0°C for 1 h (26) also resulted in the recovery of starting material.

17-Methyl-18-nor-estra-1,3,5(10),13(17)-tetraen-3-ol (3)

A solution of (2a) (150 mg) in isopropanol (10 mL) was refluxed with sodium iodide (400 mg) in an atmosphere of nitrogen for 24 h. The solvent was removed in a stream of nitrogen and then ice cold water was added. The solid was filtered and dried (145 mg). TLC of this product [silica gel-hexane-ethyl acetate (3:1)] indicated the presence of essentially one compound. Preparative TLC [silica gel; hexane-ethyl acetate (2:1)] furnished pure 17-methyl-18-norestra 1,3,5(10),13(17)-tetraen-3-ol (3) as white crystalline material mp 95°, NMR ($CDCl_3$), δ 1.65 (s,C-17 CH_3); no signal for vinylic protons.

17β-Methyl-18-nor-estra-1,3,5(10),13-tetraen-3-ol (4).

A solution of 17α-estradiol (1c) (200 mg) and TPMI (660 mg) in dry DMF (10 mL) (distilled over calcium hydride) was stirred at room temperature under N_2 for 18 h. The reaction mixture was slowly poured into ice and water (200 g). The aqueous phase was decanted from the gummy product and the residue was taken up in ethyl acetate. The ethyl acetate solution was washed with 5% sodium thiosulphate, water and dried (Na_2SO_4), and the solvent removed. The gummy residue was purified by preparative TLC (silica gel; benzene-MeOH (10:1)] and (4) (85 mg) was isolated. MP 147-148° NMR ($CDCl_3$) δ 1.01 (d,J=7 Hz, C-17 CH_3), 6.57 (s,1H,C_4-H), 6.63 (d of d,J_1=7 Hz,J_2=2 Hz, C_2-H,7.18 (1H,d,J=7 Hz,C_1-H).

3-Hydroxyestra-1,3,5(10)-triene-17-hydrazone (5b)

A mixture of estrone (5a) (1 g), triethylamine (2 mL), 95% hydrazine (5 mL) in ethanol (10 mL) was refluxed for 1.25 h and, after cooling, was poured into 200 mL of ice cold water. The crystalline hydrazone (5b) was filtered and dried (1 g). NMR δ 0.88 (s,13β-CH_3).

2,4,17-Tri-iodoestra-1,3,5(10),16-tetraene-3-ol (6a)

A solution of iodine (1.2 g) in THF (5 mL) was added dropwise at room temperature to a stirred mixture of the hydrazone (5b) (220 mg), triethylamine (1 mL) and dry tetrahydrofuran (THF) (5 mL) (27). The stirring was continued for 5 min., then most of the solvent was removed under reduced pressure and the mixture was poured into ice cold water. Following acidification (2NHCl), the products were extracted with ethyl acetate. The extract was washed with 5% aq. sodium thiosulphate, water, and dried (Na_2SO_4). Removal of the solvent in vacuo gave a gummy product (350 mg) which showed two major spots on TLC. Fractionation by preparative TLC (silica gel, hexane-ethyl acetate (3:1)] gave two crystalline products. The less polar fraction (204 mg) was characterized as 2,4,17-tri-iodoestra-1,3,5(10),16-tetraene-3-ol (6a) mp 160° (EtOH-$CHCl_3$). NMR: δ 0.75 (3H,s, C_{18}-H), 5.8 (OH, disappeared on exchange with D_2O), 6.2 (1H,t,J=2 Hz, C_{16}-H), 7.63 (1H,s,C_1-H). The more polar fraction (116 mg) was identified as 17-iodoestra-1,3,5(10),16-tetraeno-3-ol (6b) mp 146-147°.

17-Iodoestra-1,3,5(10),16-tetraene-3-ol (6b)

A solution of the hydrazone (5b) (850 mg, 3 mmole) and triethylamine (2 mL, dried over Al_2O_3) in dry THF (30 mL) was stirred under N_2. Then a solution of iodine (840 mg, 3.3 mmole) in THF (10 mL) was added dropwise and the stirring was continued for another 10 min. The mixture was poured into water, acidified with 2N HCl and 5% sodium thiosulphate (5 mL) was added. The product was extracted with ethyl acetate, washed with water, dried, (Na_2SO_4) and the solvent removed under reduced pressure to yield a fluffy solid. Purification by preparative TLC [silica gel, hexane-ethyl acetate (3:1)] furnished crystalline (6b) (850 mg) mp 147-149°; NMR δ 0.77 (3H,s,C_{18}-H), 6.21 (1H,t,J=2 Hz,C_{16}-H), 6.62 (1H,s,C_4-H), 6.68 (1H,d of d, J_1=8 Hz,J_2=2 Hz,C_2-H) and 6.88 (1H,d,J=8 Hz,C_1-H).

3,17β-Dihydroxyestra-1,3,5(10),6-tetraen-3,17-diacetate (9a)

The required 6α-hydroxy (8a) and 6β-hydroxy (8b) compounds were prepared as described by Burrows et al. (28,29).

A mixture of the 6α-hydroxy-3,17-diacetate (8a) (526 mg, 2 mmole) and TPMI (1.36 g, 3mmole) in dry DMF (15 mL) was stirred at 25° for 18h under nitrogen. The dark brown solution was then slowly added with stirring to cold water (100 mL) and the product was extracted with ether. The ether extract was washed with a 5% solution of sodium thiosulfate, followed by water, dried (Na_2SO_4) and the solvent removed. The residue, was fractionated by column chromatography (silica gel, hexane-ethyl acetate) to yield (9a) (490 mg) mp 154-56° (methanol).

Similarly, the 6β-hydroxy-3,17-diacetate (8d) on treatment with TPMI gave the (9a) in excellent yield.

When the 6-tosylates (8c) and (8e) were treated with NaI in refluxing acetone or NaI in isopropanol (25) or MgI_2 in ether (26) at 0°, only (9a) was isolated.

Estra-1,3,5(10),6-tetraene-3,17β-diol (9b), mp 220-222°C was prepared by treating (9a) (150 mg) with $LiAlH_4$ (120 mg) in ether (5 mL).

17α-Iodoestra-1,3,5(10),6-tetraene-3-ol (9c)

A mixture of 6-dehydroestradiol (9b) (115 mg), TPMI (450 mg) and DMF (3 mL) was stirred under nitrogen for 16 h. After a conventional workup, the residue was purified first by column chromatography and then by preparative TLC [silica gel, hexane-ethyl acetate (3:1)] and the 17α-iodide (9c) (80 mg) mp 121-123° (dec) was obtained. NMR ($CDCl_3$) δ 0.86 (3H,s,C_{18}-H), 4.43 (1H,d of d,J_1=7 Hz,J_2=2 Hz,17β-H). The 17α-iodide (9c) was also obtained by treating the 6α-hydroxyestradiol (8b) with TPMI in DMF as described earlier.

Estra-1,3,5(10)-triene-3,17β-diol-6-hydrazone (7b)

A mixture of the 6-ketoestradiol-3,17β-diacetate (7a) (39,40) (370 mg) triethylamine (0.8 mL), 95% hydrazine (1.0 mL) and absolute ethanol (5 mL) was refluxed for 1.25 h. The cooled mixture was poured into cold water. The crystalline hydrazone (7b) (285 mg) was filtered, dried, and the crude product was used in subsequent reactions.

2,6-Diiodoestra-1,3,5(10),6-tetraene-3,17β-diol (10a) and 6-iodoestra-1,3,5(10),6-tetraene-3,17β-diol (10b)

A solution of the hydrazone (7b) (225 mg, 0.75 mmole) and triethylamine (dried over Al_2O_3) (0.5 mL) in dry THF (freshly distilled over LAH) (5 mL) was stirred under nitrogen. Then a solution of iodine (256 mg, 1 mmole) in dry THF (5 mL) was added dropwise, until the developing pink color persisted. When the evolution of nitrogen ceased (10-15 min.), the mixture was poured into ice cold water and acidified with 2N.HCl. A solution of 5% sodium thiosulfate was added dropwise to discharge the color of iodine and then the products were recovered with ethyl acetate. The extract was processed in the conventional manner to yield a gummy residue (340 mg). The residue was fractionated by preparative TLC [silica gel, developed first with hexane-ethyl acetate (2:1) and then with hexane-ethyl acetate (1:1)] to give two crystalline compounds. The less polar material was found to be 2,6-iodide (10a) (75 mg) mp 146-50° (dec); NMR: 0.77 (s,C_{18}-H), 3.8 (t,J=8

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[1] C. Longcope, T. Arunachalam, and E. Caspi, unpublished observations.

[2] The abbreviations used are; NIS, N-Iodosuccinimide; TPMI, Triphenylphosphite Methiodide; THF, Tetrahydrofuran; Me_2SO, Dimethyl Sulfoxide; NMR, Nuclear Magnetic Resonance; IR, Infrared; Ms, Mass Spectrum; Ac, Acetate; Ts, p-toluenesulfonate; DDQ, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone; MeOH, Methanol; Hz, Hertz; TLC, Thin Layer Chromatography; DMF, Dimethylformamide; HMPT, Hexamethylphosphorous Triamide; LAH, Lithium Aluminum Hydride; ^3H-E_2, [2,4,6,7-^3H]-estra-1,3,5(10)-triene-3,17β-diol; HOI, Hypoiodous Acid.

Hz,17α-H) 6.8 (bs,C_7-H), two singlets at 7.16 and 7.47 (C_1-H) & C_4-H), 1.67 (C_{17}-OH) and 5.3 (phenolic OH). Mass spectrum: m/e 522 (M^+). (Calc'd for $C_{18}H_{20}O_2I_2$; 522). The major, more polar product was 6-iodoestra-1,3,5(10),6-tetraene-3,17β-diol (10b) (130 mg) mp 137-40° (dec). NMR: δ 6.73 (s,C_{18}-H), 3.7 (t,J=8 cps,17α-H). Mass spectrum: m/e 396 (M^+), (Calc'd for $C_{18}H_{21}O_2I$; 396). The formation of the diiodide (10a) could be avoided by slow, dropwise addition of 0.5 equivalent of iodine (solution). The color of the mixture should be completely discharged before the next drop of the iodine solution is added. Under these conditions the 6-iodide (10b) was formed in about 40% yield.

Hydrogenation of the vinyl iodides (10a) and (10b) over 5% Pd/C in ethyl acetate resulted in the loss of iodine.

7α-Iodo-3,6β,17β-trihydroxyestra-1,3,5(10)-triene-3,17-diacetate (11a)

A solution of the diacetate (9a) (90 mg, 25 mmole) and N-iodosuccinamide (80 mg, 0.35 mmole) in acetone (5 mL) was stirred and cooled to 5°. Then 0.2N $HClO_4$ (0.41 mL) was added during 15 min. and the stirring (at 5°) was continued for an additional 30 min. Aqueous sodium thiosulphate (10%) was added to the mixture until the KI-starch paper test was negative. Water (30 mL) was added and the product was recovered with ether. The ether solution was washed, dried (Na_2SO_4) and removal of the solvent gave a solid. Crystallization from EtOAc-hexane furnished white crystals of the iodohydrin (11a) (100 mg), mp 161° (dec). NMR δ 0.9 (s,C_{18}-H), 2.07 (s,17-OAc), 2.3 (s,3-OAc), 4.43 (1H,7β-H), 4.75 (t,J=7 Hz,17β-H), 5.05 (d,J=1.5 Hz,6α-H).

7α-Iodoestra-1,3,5(10)-triene-3,6β,17β-triol (11b)

A solution of 6-dehydroestradiol (9b), (80 mg, 0.3 mmole) and N-iodosuccinimide (91 mg, 0.4 mmole) in acetone (6 mL) was cooled to (5°C), then aqueous 0.2N $HClO_4$ (0.5 mL) was added dropwise (15 min). After stirring at 5° for 30 min, the reaction was worked up as described above. The crude product was fractionated by preparative TLC [silica gel, hexane-EtOAc-MeOH (10:9:1)] to furnish 7α-iodotriol (11b) (120 mg) mp 135-137°C (hexane-ethyl acetate) NMR ($CDCl_3$-DMSO-d_6) δ 0.83 (s,C_{18}-H), 3.72 (t,J=8 Hz,17α-H), 4.47 (bs,C_7-βH), 4.91 (bs,6α-H), 6.77 (d,J=8 Hz, C_2-H), 6.83 (bs,C_4-H), 7.2 (d,J=8 Hz,C_1-H).

7α-Iodo-6-ketoestra-1,3,5(10)-triene-3,17β-diacetate (12)

Jones' reagent (0.2 mL) was added to a stirred and cooled (0°) solution of the iodohydrin (11a) (50 mg) in acetone (2 mL). Stirring was continued for 30 min. and the excess reagent was destroyed with methanol. The solvent was removed in a stream of nitrogen, cold water was added, and the crystalline 6-ketone (12) was filtered and dried (45 mg). IR (KBr): ν_{max} 1754 (3-Ac), 1724 (17-OAc) and 1675 cm^{-1} (6-ketone). NMR ($CDCl_3$) δ 0.88 (s,C_{18}-H), 2.07 (s,17-OAc), 2.32 (s,3-OAc), 4.78 (d,J=2 Hz,7β-H), 4.7 (t,17α-H).

3-Hydroxyestra-1,3,5(10),9(11)-tetraen-17-one (13)

A solution of 11α-hydroxyestrone (15) (31) (72 mg, 0.25 mmole) and TPMI (0.4 mmole) in dry DMF (5 mL) was stirred for 18 h, under nitrogen atmosphere. Following the usual workup and purification by preparative TLC [silica gel, hexane-ethyl acetate (2:1)], $\Delta^{9(11)}$-estrone (13) (45 mg) mp 246-48° (241-47° lit. 33,34) was obtained. NMR δ 0.93 (s,3H,18-H), 6.07 (broad peak, C_{11}-H).

Authentic samples of (13) were prepared in 40% yield by treatment of estrone (5) with fluorosulphonic acid in the presence of antimony pentafluoride (32). Alternatively, exposure of estrone to 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in methanol or dioxane (33,34) also gave the olefin (13).

3-Hydroxy-4-iodoestra-1,3,5(10),9(11)-tetraen-17-one (14)

A solution of 3-hydroxy-estra-1,3,5(10),9(11)-tetraen-17-one (13) (95 mg) and N-iodosuccinimide (110 mg) in acetone (8 mL) was stirred at -5° and then 0.2N perchloric acid (0.6 mL) was added dropwise during 15 min. The stirring was continued for 30 min. at -5°C. Subsequently, a 10% solution of sodium thiosulfate was added until a KI-starch paper test was negative and the mixture was diluted with water.

The product was recovered (ethyl acetate) and processed in the usual manner to give a semi-solid. Crystallization from ethyl acetate furnished 4-iodo-3-hydroxy-estra-1,3,5(10),9(11)-tetraen-17-one (14) [15 mg, mp 155-156° (dec)]. TLC of the mother liquid [hexane-ethyl acetate (1:1)] gave an additional amount of the iodide 14 [95 mg, mp 159-160° (methanol)]. NMR: δ 0.92 (3H,s,C_{18}-H), 6.1 (broad peak,C_{11}-H), 6.8 (1H,d,J=8 Hz,C_2-H), and 7.4 (1H,d,J=8 Hz,C_1-H). Mass spectrum: m/e at 394 (M^+) (calc'd: $C_{18}H_{20}O_2I$,394).

16β-Iodoestra-1,3,5(10)-triene-3,17β-diol (17)

A solution of estriol (16) (145 mg, 0.45 mmole) and TPMI (455 mg, 1 mmole) in dry DMF (3 mL) was stirred under nitrogen (20 h). The dark pink mixture was added dropwise, with stirring, to ice and water (100 mL). The product was recovered with ethyl acetate, the extract was washed with 5% sodium thiosulfate, water and dried (Na_2SO_4). Removal of the solvent gave a gummy residue which was resolved by preparative TLC [silica gel, hexane-ethyl acetate (2:1)] into five fractions (listed in order of increasing polarity).

Fraction #1: Contained traces of material and was not investigated.

Fraction #2: Yielded estra-1,3,5(10),16-tetraen-3-ol (18a) (40 mg) mp 129-30° NMR ($CDCl_3$): δ 0.85 (18-H), 5.92 (2H,m,16- and 17-H), 6.68 (s,C_4-H), 6.75 (d of d,J_1=8 Hz,J_2=2.5 Hz,C_2-H) and 7.27 (d,J=8 Hz,C_1-H).

Fraction #3: Gave the phosphonate ester (18b) (10 mg). NMR: 1.78 (3H,d,J=18 Hz,P-CH_3), 5.88 (2H,m,C_{16}- and C_{17}-H) and signals for eight aromatic protons.

Fraction #4: The residue (40 mg) was crystallized from hexane-ethyl acetate to give 16β-iodo-estra-1,3,5(10)-triene 3,17β-diol (17): at 110-120°, the color of the crystals changed to pink and then again to white which melted at 215-30°. The product (17) gave a strong positive Beilstein test. NMR: ($CDCl_3$: DMSO-d_6) δ 0.96 (3H,s,C_{18}-H), 4.75 (2H,m,16α- and 17α-H), 6.57 (s,C_4-H), 6.64 (d of d,J_1=8 Hz,J_2=2.5 Hz,C_2-H) and 7.1 (d, J=8 Hz,C_1-H). Mass spectrum: m/e at 398 (M^+), 270 (M^+-HI) (calc'd for $C_{18}H_{23}O_2I$,398).

Treatment of (17) (15 mg) with 5% methanolic sodium hydroxide gave estrone (9 mg).

Fraction #5: Gave diphenyl methyl phosphonate (CH_3-P(O)-(OPh)$_2$) (160 mg,oil).

When excess of TPMI (≥ 4 molar equivalents) was used, the major product of the reaction was the Δ^{16}-olefin (18a) (60-70% yield). When equimolar amounts of TPMI and (16) were used, the major product was (17).

17α-Iodoestra-1,3,5(10)-triene-3,16β-diol (19a)

N-iodosuccinimide (NIS) (80 mg, 0.35 mmole) was added to a cooled solution (-5° to 0°) of estra-1,3,5(10),16-tetraen-3-ol (18a) (64 mg, 0.25 mmole) in acetone (AR grade, 4 mL). To the stirred mixture, 0.2N perchloric acid (0.41 mL) was added dropwise (10-15 min.). The reaction was carried out under nitrogen, and after stirring for 30 min. at (-5° to 0°) a 10% solution of sodium thiosulfate was added until the KI-starch paper test was negative. The reaction mixture was then diluted with water (25 mL) and the product extracted with ethyl acetate. The extract was washed with water, dried (Na_2SO_4) and the solvent removed under reduced pressure to yield a gummy product. The residue was fractionated by preparative TLC [silica gel, hexane-ethyl acetate (3:2)] into one major and two minor zones. Extraction of the major band (low R_f value) furnished crystalline 17α-iodoestra-1,3,5(10)-triene-3,16β-diol (19a) [72 mg,75% mp 164-68° (dec)]; Beilstein test positive; NMR ($CDCl_3$ + DMSO-d_6): δ 1.17 (3H,s,18-H), 4.31 (1H,bs,17β-H), 5.16 (1H,m,16α-H), 6.73 (1H,s,C_4-H), 6.8 (1H,d of d,$J_{2,1}$=8 Hz,$J_{2,4}$=2 Hz,C_2-H), and 7.34 (1H,d,$J_{1,2}$=8 Hz,C_1-H).

From the two minor bands 2,4,17α-triiodoestra-1,3,5(10)-triene-3,16β-diol (19b) and 4,17α-diiodoestra-1,3,5(10)-triene-3,16β-diol (19c) were recovered in about 5% yield.

17α-Iodomethyl estra-1,3,5(10)-triene-3,17β-diol (21)

A 47% solution of hydroiodic acid (36) (0.5 mL) was added dropwise to a stirred solution of the 17β-oxirane 20 (35) (50 mg) in dioxane (2 mL) and the stirring was continued for 30 min. The dark pink solution was diluted with ethyl acetate. The ethyl acetate solution was washed successively with a 5% solution of sodium thiosulfate, 2% solution of sodium bicarbonate, water, and dried (Na_2SO_4). Removal of the solvent gave a gummy residue. The product was purified by chromatography over a column of silica gel. Elution with hexane-ether (3:1) furnished crystalline 17α-iodomethyl estradiol (21) (45 mg). mp 146-51° (from ethyl acetate-hexane). NMR ($CDCl_3$): δ 1.03 (3H,s,18-H), 3.6 (2H,AB quartet, δ_{AB}=16 Hz,J_{AB}=10 Hz,CH_2-I).

16α-Iodo-3-hydroxyestra-1,3,5(10)-trien-17-one (22b)

The acetate (22a) (mp. 163-165°C) was prepared (37) and hydrolysed with methanolic HCl to give the crystalline 16α-iodoestrone (22b) (145 mg). Recrystallization from methanol gave (22b) mp. 210-211° C (dec) (lit 213° (dec)) NMR δ 0.92 (s,3H,18-H), 4.93 (broad peak, 16β-H).

2-Iodo-estra-1,3,5(10)-triene-3,17β-diol (23)

The product was prepared as described by Hillmann-Elies et al. (38) and showed mp 127-28° (mp 129-30°). NMR ($CDCl_3$-DMSO-d_6) δ 0.77 (3H,s, 18-H), 3.7 (1H,t,J=7 Hz,17α-H), 6.69 (s,1H,C_4-H) and 7.6 (s,1H,C_1-H).

Biological Evaluation of Iodo-Estrogens

Preparation of Cytosol: For studies using the dextran coated charcoal assay or sucrose gradients, cytosol from rabbit uterus was prepared according to the published method (41,42). Uteri from mature virgin female rabbits were removed, cleaned and frozen in liquid nitrogen. The frozen uteri were then pulverized, using a thermovac pulverizer (purchased from Thermovac Ind., Copiague, NY), homogenized (2:1, wt/vol) in Tris buffer (Tris:HCl,0.01 M; pH 7.4; 0.0015 M EDTA; and 0.5 mM Dithiothreitol) 2:1 (vol/wt) using a polytron SW-10 homogenizer in three 10 sec bursts. The homogenate was centrifuged at 4° at 140,000 x g for 50 min, and then the supernatant used as described below.

Dextran Coated Charcoal Assay: The dextran coated charcoal assay for displacement of [^{3}H]estradiol by iodinated steroids was carried out as previously described (43). To a series of 12 x 75 mm tubes were added incremental amounts of either nonradioactive estradiol or nonradioactive iodinated steroid in ethanol. The ethanol was removed under N_2 and to the tubes were added 10,000 dpm of 2,4,6,7-[^{3}H]estradiol (sp. ac. 91.8 Ci/Millimole) in 200 µl buffer and 20 µl of the cytosol. The tubes were incubated for 16 h at 4°C and, following the incubation, 1 mL of dextran coated charcoal (0.25% Norit A, 0.025% dextran in Tris buffer) was added. The tubes were then centrifuged at 4°C at 3,000 rpm. The supernatant was removed, placed into counting vials to which was added 5 mL Aquasol (purchased from New England Nuclear Co., Boston, MA). The radioactivity was measured in a Packard liquid scintillation spectrometer. The results were then plotted at B/B_0 vs. amount of added steroid (43), and the relative binding affinity of the iodinated estrogens compared to estradiol was calculated (44).

Inhibition Experiments: Certain iodinated steroids which showed inhibition of [^{3}H]estradiol binding were further studied to determine the type of inhibition. To all but one of a series of tubes was added iodinated steroid at three concentrations in ethanol. The ethanol was removed under nitrogen and to all the sets of tubes were added incremental amounts of [^{3}H]estradiol in buffer and 20 µl of cytosol. The tubes were incubated overnight at 4°C. Following the incubation, 0.5 mL dextran-coated charcoal solution was added to all tubes, the contents of which were mixed mechanically and allowed to incubate for 20 min. at 4°C. Following the incubation with dextran-coated charcoal all tubes were centrifuged at 3,000 rpm at 4°C. The supernatant was removed into counting vials, 5 mL Aquasol was added and the radioactivity measured in a liquid scintillation spectrophotometer. The data was analyzed for free and bound steroid and plotted, using a Scatchard type plot (45). The number of sites and the K_d were then calculated.

Sucrose Gradients: Sucrose gradients, 10-30% (41) were used. Uterine cytosol, 250 µl, was incubated for 15 min. with 1.0 µl of buffer containing varying amounts of iodinated estrogen or estradiol. Then, 1 picomole of [^{3}H]estradiol was added and the tubes incubated for 4 h at 4°C. Following incubation, the cytosol was removed and incubated for 20 min. over a charcoal pellet at 4°C. Following the second incubation, the charcoal was removed by centrifugation at 3200 rpm. The supernatant, 200 µl, was layered on sucrose gradients and 10 µl of 4-^{14}C-albumin (46) in buffer added as an internal marker. The tubes were centrifuged at 4°C at 297,000 X g for 16 1/3 h. Following centrifugation, 0.1 mL fractions were collected in vials. Five mL Aquasol was added and the radioactivity determined in a liquid scintillation spectrometer. The counts per minute were then plotted against tube number. The 8S and 4S peaks were determined and the degree of displacement by the iodinated estrogen calculated.

In Vivo Studies: Twenty-one day old female rats maintained on regular food and aqueous .03 mM KI, ad lib, were injected with [^{3}H]estradiol, 2 µCi, and varying quantities of suspending vehicle or of the tested iodinated estrogen. Two hours later the rats were sacrificed, the uterus was removed, cleaned, weighed and solubilized, using Protosol (1.5 mL) tissue solubilizer (purchased from New England Nuclear, Boston, MA). Aquasol (5 mL) was added and the radioactivity determined in a liquid scintillation spectrometer.

Results

Dextran Coated Charcoal Assay: The relative binding affinities are noted in Tables 1 and 2. Of the compounds tested not one with an iodine atom at the 17α or at the 2 position was active in displacing the [^{3}H]estradiol from the cytosol receptors. The relative binding activities being 1% or less for these compounds. The 16α-iodoestrone (22b), 16β-iodoestradiol (17), 6-iodoestra-1,3,5(10),6-tetraene-3,17β-diol (10b), 7α-iodoestra-1,3,5(10)-triene-3,6β,17β-triol (11b) and the 4-iodoestra-1,3,5(10),9(11)-tetraen-3-ol-17-one (14) all had binding affinities relative to estradiol above 0.01 with the greatest binding affinity 0.57 being noted for the 16β-iodoestradiol (17).

Inhibition studies were carried out using 3 of the most active iodinated estrogens. These results are noted in Figure 1. It is seen that for these three iodinated compounds the addition of increasing amounts of the iodinated estrogen resulted in a shift of the binding affinity for the [^{3}H]estradiol but not in the number of sites. This is compatible with competitive inhibition as the cause for the displacement of the [^{3}H]estradiol.

Sucrose Gradients: As noted in Figure 2 there was displacement of [^{3}H]estradiol from the 8S and 4S peaks of receptor by the 3 iodinated estrogens. This displacement was complete at concentrations down to 40 nanomolar.

In Vivo Administration: As shown in Figure 3, all 3 iodinated estrogens resulted in inhibition of [^{3}H]estradiol binding by the rat uterus in vivo. The most active agent being 16β-iodo-estradiol (17) with the Δ^6-6-iodo-estradiol (10b) less potent and the 7-iodide (11b) being the weakest inhibitor of [^{3}H]estradiol binding.

References

1. Jensen, E. V., Block, G. R., Smith, S., and De Sombre, E. R. (1973) in "Breast Cancer: A Challenging Problem" (Griem, M. L., Jensen, E. V., Ultmann, J. E., and Wissler, R. W., Eds) p 55, Springer-Verlag, Berlin.

2. McGuire, W. L., Horwitz, K.B., Zava, D. T., Garola, R. E., and Chamness, G. C. (1978) Metabolism 27, 487-501.

3. Estrogen Receptors in Human Breast Cancer (1975) (McGuire, W. L., Carbone, P. P., and Vollmer, E. P., Eds) Raven Press, New York.

4. Spitznagle, L. A., and Marino, C. A. (1977) 173rd National Meeting of the American Chemical Society, Abst. #36.

5. Chambers, V. E. M., Glover, J. S., and Tudor, R. (1975) in: Steroid Immunoassay, Proc. Fifth Tenovus Workshop, Cardiff, April 1974, Cameron, E. H. D., Hillier, S. C., and Griffiths, Ed., Alpha Omega Publ. Ltd., pp. 177-192.

6. Counsell, R. E., Hong, B. H., Willette, R. E., and Ranade, V. V. (1968) Steroids 11, 817-826.

7. Szendröi, Z., Kocsár, L., Karika, Z., and Eckhardt, S. (1973) Lancet June 2, 1252-1253.

8. Katzenellenbogen, J. A., Hsiung, H. M., Carlson, K. E., McGuire, W. L., Kraay, R. J., and Katzenellenbogen, B.S. (1975) Biochemistry 14, 1742-1750.

9. Katzenellenbogen, J.A., and Hsiung, H. M. (1975) Biochemistry 14, 1736-1741.

10. Szendröi, Z., Kocsár, Töttösy, B., Karika, Zs., and Eckhardt, S. (1975) Neoplasma 22, 535-537.

11. Ghanadian, R., Waters, S. L., Thakur, M. L., and Chisholm, G. D. (1975) Internat. J. Appl. Radiation & Isotopes 26, 343-346.

12. Counsell, R. E., Buswink, A., Korn, N., Johnson, M., Ranade, V., and Yu, T. (1976) in: Steroid Hormone Action and Cancer, Menon, K. M. J., and Reel, J. R., eds. Plenum Press, pp. 107-113.

13. Komai, T., Eckelman, W.C., Johnsonbaugh, R. E., Mazaitis, A., Kubota, H., and Reba, R. C. (1977) J. Nucl. Med. 18, 360-366.

14. Katzenellenbogen, J. A. (1977) in "Biochemical Actions of Hormones" (Litwack, G., Ed) Chapter 1, pp. 1-84, Academic Press, New York.

15. Martucci, C., and Fishman, J. (1976) Steroids 27, 325-333.

16. Katzenellenbogen, J. A. (1974) in "Ann. Reports in Medicinal Chemistry" (Heinzelman, R. V., Ed) Vol. 9, Chapter 23, pp. 222-233, Academic Press, New York.

17. Chin, C-C., and Warren, J. C. (1968) J. Biol. Chem. 243, 5056-5062.

18. Ganguly, M., and Warren, J. C. (1971) J. Biol. Chem. 246, 3646-3652.

19. Sweet, F., Arias, F., and Warren, J.C. (1972) J. Biol. Chem. 247, 3424-3433.

20. Korenman, S. G. (1969) Steroids 13, 163-177.

21. King, R. J. B., and Mainwaring, W. I. P. (1974) Steroid-Cell Interactions, pp. 219-220, Park Press, Baltimore, MD.

22. Landauer, S. R., and Rydon, H. N. (1953) J. Chem. Soc., 2224-2234.

23. Verheyden, J., and Moffatt, J. (1970) J. Org. Chem. 35, 2319-2326.

24. Counsell, R. E., Kulkarni, P. G., Fiatpour, P. A., and Ranade, V. V. (1973) J. Med. Chem. 16, 945-947.

25. Counsell, R. E., Ranade, V. V., Blair, R. J., Beierwalters, W. H., and Weinhold, P. A. (1970) Steroids 16, 317-328.

26. Core, J., Place, P., and Roumestrant, M. L. (1973) J. Chem. Soc. Chem. Commun., 821-823.

27. Barton, D. H. R., O'Brien, R. E., and Sternhell, S. (1962) J. Chem. Soc., 470-476.

28. Burrows, E. P., Jones, S. L., Smith, H. E. (1973) J. Org. Chem. 38, 3797-3798.

29. Burrows, E. P., Jones, S. L., Smith, H. E. (1972) J. Org. Chem. 37, 4000-4002.

30. Tsuda, K., Nozoe, S., and Okada, Y. (1963) Chem. Pharm. Bull. (Japan) 11, 1022-1027.

31. Baran, J. S. (1967) J. Med. Chem. 10, 1188-1190.

32. Gresson, J. P., Jacquesy, J. C., and Jacquesy, R. (1971) Tetrahedron Lett., 4733-4736.

33. Cross, A.D., Carpio, H., and Crabbe, P. (1963) J. Chem. Soc., 5539-5544.

34. Osawa, Y., Japanese Patent 6800508, Chem. Abs 69, 87329K.

35. Cook, C.E., Corley, R. C., Wall, M. E. (1968) J. Org. Chem. 33, 2789-2793.

36. Fajkos, J., Joska, J., and Sorm, F. (1962) Coll. Czech. Chem. Comm. 27, 64-72.

37. Mueller, G. P., Johns, W.F. (1961) J. Org. Chem. 26, 2403-2413.

38. Hillmann-Elies, V. A., Hillmann, G., Schiedt, V. (1953) Zeit. Natur. 86, 436-439.

39. Longwell, B., Wintersteiner, O. (1940) J. Biol. Chem. 133, 219-229.

40. Combie, R. C., Mander, L. N., Bose, A. K., and Manhas, M. S. (1964) Tetrahedron 20, 409-416.

41. McGuire, W. L. (1973) J. Clin. Invest. 52, 73-77.

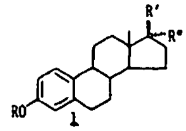
42. McGuire, W. L., and DeLaGarza, M. (1973) J. Clin. Endocrinol. Metab. 37, 986-989.

43. Nagai, N., and Longcope, C. (1971) Steroids 17, 631-647.

44. Rodbard, D. (1973) Adv. Exptl. Med. Biol. 36, 289-326.

45. Scatchard, G. (1949) Ann. N. Y. Acad. Sci. 51, 660-672.

46. Rice, R. H., and Means, G. E. (1971) J. Biol. Chem. 246, 831.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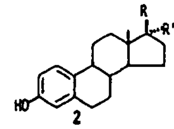
b. R=Ac; R'=OAc; R"=H
c. R=H; R'=H; R"=OH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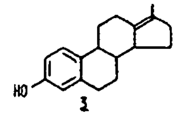
a. R=H; R'=I
b. R=I; R'=H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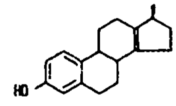
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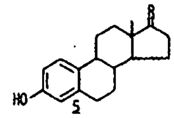
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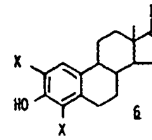
a. R=O
b. R=H,NH_2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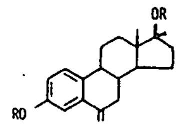
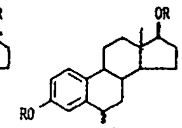
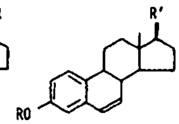
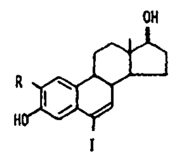
a. X=I
b. X=H


7
a. R = Ac R' = O
b. R = H; R' = H,NH_2


8
a. R = Ac; R' = α-OH
b. R = H; R' = α-OH
c. R = Ac; R' = α-OTs
d. R = Ac; R' = β-OH
e. R = Ac; R' = β-OTs


9
a. R=Ac; R'=OAc
b. R=H; R'=OH
c. R=H; R'=α-I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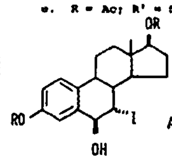
a. R=I
b. R=H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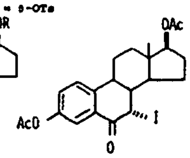
a. R=Ac
b. R=H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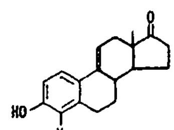
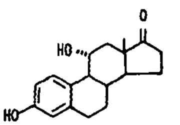

13 X=H
14 X=I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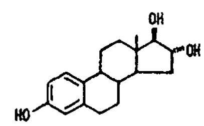
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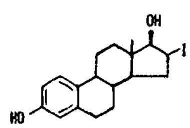
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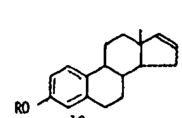
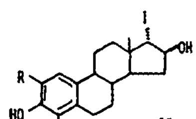
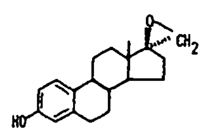

18
a. R=H
b. R=P(=O)(CH_3)OC_6H_5


19
a. R=R'=H
b. R=R'=I
c. R=H; R'=I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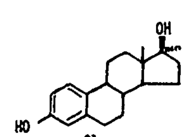
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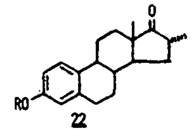
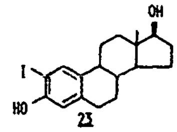

22
a. R=Ac
b. R=H


23

RÉPUBLIQUE FRANÇAISE

MINISTÈRE DE L'INDUSTRIE

SERVICE
de la PROPRIÉTÉ INDUSTRIELLE

BREVET SPÉCIAL DE MÉDICAMENT

P.V. n° 45.659 N° 5.099 M

Classification internationale : A 61 k // C 07 c

Nouveau médicament notamment pour le traitement de l'hypercholestérolémie et des troubles en résultant.

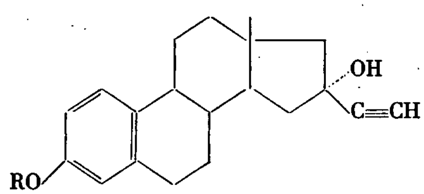
Société dite : ROUSSEL-UCLAF résidant en France (Seine).

Demandé le 12 janvier 1966, à 15^h 25^m, à Paris.

Délivré par arrêté du 22 mai 1967.

(*Bulletin officiel de la Propriété industrielle* [*B.S.M.*], n° 26 du 26 juin 1967.)

La présente invention a pour objet, à titre de nouveau médicament, notamment pour le traitement de l'hypercholestérolémie et des troubles en résultant, le 3,16α-dihydroxy 16β-éthynyl estra-1,3,5(10)-triène, ses éthers et ses esters, de formule :



dans laquelle R représente de l'hydrogène, un radical alcoyle ou un reste d'acide organique carboxylique, conditionnés en vue de l'usage au poids médicinal et les compositions en renfermant.

Le 3,16α-dihydroxy 16β-éthynyl estra-1,3,5(10)-triène (R = H) se présente sous forme d'un produit solide incolore, soluble dans l'alcool, insoluble dans l'eau et le propylène-glycol.

Son point de fusion, déterminé sur bloc de Kofler, est de F = 215 °C. Son pouvoir rotatoire est de $[\alpha]_D^{20} = +47{,}5$ °C ($c = 0{,}7$ %, dioxane). Le spectre I.R. (chloroforme) montre :

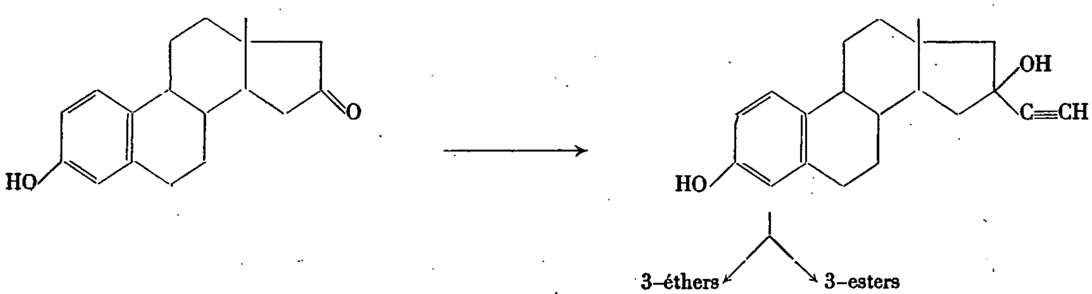
Absorptions aromatiques à 1 570 et 1 620 cm^{-1};

Hydroxyle très associé, mais absence d'une bande d'absorption à 3 480 cm^{-1} comme le composé 16α-éthynyl 16β-hydroxylé;

Une bande éthynyl à 2 125 cm^{-1}.

Le principe de la préparation consiste en ce que l'on fait agir l'acétylure de potassium sur le 3-hydroxy 16-oxo estratriène en présence de dioxane, obtient le 3,16α-dihydroxy 16β-éthynyl estra-1,3,5(10)-triène que l'on estérifie ou éthérifie si désiré par les méthodes classiques.

Le schéma suivant explicite la réaction :



Mode opératoire.

1° *Préparation de l'acétylure de potassium.* — On chauffe à 60 °C un mélange de 260 cm^3 d'alcool teramylique et de 90 cm^3 de benzène, ajoute lentement, sous agitation, 23,5 g de potassium et maintient l'agitation deux heures à 65 °C; on ramène à la température ambiante, ajoute 100 cm^3 de dioxane et on fait passer un courant d'acétylène pendant deux heures trente minutes.

2° *Ethynylation.* — A la solution d'acétylure de potassium obtenue ci-dessus, on ajoute une solution de 3,9 g de 3-hydroxy 16-oxo estra-1,3,5(10)-triène dans 120 cm^3 de dioxane; on agite à 32-33 °C pendant quatre heures, en maintenant le courant d'acétylène, refroidit, ajoute un excès de solution saturée de chlorure d'ammonium, extrait au benzène, lave les phases organiques à l'eau et évapore à sec sous vide; on recueille 5,6 g de composé, on le chromatographie d'abord sur silice, puis après cristallisation d'une partie du produit, on soumet les eaux mères à une nouvelle chromatographie sur alumine, ce qui permet d'isoler une nouvelle quantité de produit

attendu, les deux chromatographies étant effectuées dans le mélange benzène-acétate d'éthyle (7 : 3). On termine par une recristallisation dans l'éther et obtient 528 mg de 3,16α-dihydroxy 16β-éthynyl estra-1,3,5(10)-triène, sous forme d'un produit solide incolore, fondant à 215 °C; son α_D est de + 47,5 °C (c = 0,7 %, dioxane).

Analyse ($C_{20}H_{24}O_2$ = 296,39).

Calculé (%) :

C : 81,04; H : 8,16.

Trouvé (%) :

C : 81,0; H : 8,5.

Spectre U.V. (éthanol) :

Infl. vers 218 mμ : $E_{1\,cm}^{1\,\%}$ = 254;

Infl. vers 221 mμ : $E_{1\,cm}^{1\,\%}$ = 246;

Infl. vers 229 mμ : $E_{1\,cm}^{1\,\%}$ = 178;

Max. à 281mμ : $E_{1\,cm}^{1\,\%}$ = 69,6;

Infl. vers 287 mμ : $E_{1\,cm}^{1\,\%}$ = 62,8.

Ce produit n'est pas décrit dans la littérature.

Le produit de départ, le 3-hydroxy 16-oxo estra-1,3,5(10)-triène est obtenu selon le procédé décrit par M. N. Huffman et M. H. Lott, *J. Am. Chem. Soc., 75*, 4327 (1953).

Le produit est doté de propriétés pharmacologiques intéressantes. Il possède notamment une action hypocholestérolémiante importante.

Il peut être utilisé pour le traitement de l'hypercholestérolémie et, comme agent préventif ou curatif des maladies artérielles, artérites cérébrales, aortites, coronarites, angine de poitrine, athéromatose.

Le 3,16α-dihydroxy 16β-éthynyl estra-1,3,5(10)-triène, ses éthers ou ses esters sont utilisés par voie buccale, perlinguale, transcutanée ou rectale.

Ils peuvent se présenter sous forme de solutions ou de suspensions injectables, conditionnées en ampoules, en flacons à prises multiples; de comprimés, de comprimés enrobés, de comprimés sublinguaux, de capsules et de suppositoires.

La posologie utile s'échelonne entre 4 et 20 mg par jour chez l'adulte en fonction de la voie d'administration.

Les formes pharmaceutiques telles que : solutions ou suspensions injectables, comprimés, comprimés enrobés, comprimés sublinguaux, capsules et suppositoires, sont préparées selon les procédés usuels.

Etude pharmacologique du médicament objet de l'invention.

1° *Action hypocholestérolémiante chez le rat femelle normal.* — On opère sur des groupes de rats femelles, d'un poids moyen de 200 g, auxquels on administre, par voie orale, le 3,16α-dihydroxy 16β-éthynyl estra-1,3,5(10)-triène utilisé en suspension dans un liquide dispersif aqueux, aux doses quotidiennes de 2 et 5 mg/kg pendant dix jours. Un groupe de rats femelles de mêmes âge et poids sert de témoin. Des prises de sang sont effectuées le onzième jour, en vue de déterminer le taux des stérols sériques. Les animaux sont sacrifiés le même jour. Les organes suivants : foie et surrénales sont prélevés et pesés.

Le tableau ci-après résume les résultats obtenus :

Lots	Doses quotidiennes	Stérols sériques	Surrénales	Foie	Prise de poids corporel
		g ‰	mg	g % g	
Témoins	0	0,70	57,5	4,33	+ 10 %
3,16α-dihydroxy 16β-éthynyl estra-1,3,5(10)-triène	2 mg/kg	0,61 (– 13 %)	58,9	4,49	+ 7 %
	5 mg/kg	0,42 (– 40 %)	71,2 (+ 24 %)	5,06	+ 10 %

Dans les conditions de l'expérience, on constate donc que le 3,16α-dihydroxy 16β-éthynyl estra-1,3,5(10)-triène a une nette action hypocholestérolémiante à la dose quotidienne de 5 mg/kg.

2° *Recherche de l'activité estrogène.* — Le 3,16α-dihydroxy 16β-éthynyl estra-1,3,5 (10)-triène a été administré en suspension aqueuse, par voie orale ou par voie sous-cutanée en une seule fois, en solution dans l'huile d'olive, à des rates castrées.

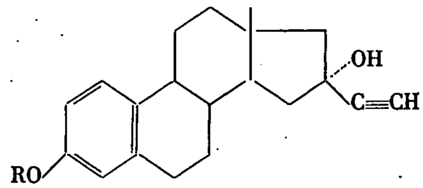
Les frottis vaginaux ont été effectués chaque jour, quarante-huit heures après le traitement et pendant cinq jours consécutifs, en ne retenant comme positifs que les frottis formés exclusivement de cellules kératinisées.

Dans les conditions de l'expérience, l'unité-rat a été trouvée supérieure à 10 mg par voie orale et à 2 mg par voie sous-cutanée. Le produit étudié n'a donc qu'une très faible activité estrogène.

RÉSUMÉ

L'invention a pour objet, à titre de nouveau médicament, notamment pour le traitement de l'hypercholestérolémie et des troubles en résultant :

1° Le 3,16α-dihydroxy 16β-éthynyl estra-1,3,5(10)-triène, ses éthers et ses esters, de formule :



dans laquelle R représente de l'hydrogène, un radical alcoyle ou un reste d'acide organique carboxylique :

F = 215 °C
$[\alpha]_D^{20} = +47,5°$ ($c = 0,7$ %, dioxane) } R = H

conditionnés en vue de l'usage au poids médicinal;

2° Le 3,16α-dihydroxy 16β-éthynyl estra-1,3,5-(10)-triène, ses éthers et ses esters présentés notamment sous forme de : solutions ou suspensions injectables, conditionnées en ampoules, en flacons à prises multiples, comprimés, comprimés enrobés, comprimés sublinguaux, capsules et suppositoires.

Société dite : ROUSSEL-UCLAF

AVIS DOCUMENTAIRE SUR LA NOUVEAUTÉ

Documents susceptibles de porter atteinte à la nouveauté du médicament : *néant.*

Documents illustrant l'état de la technique en la matière :

— *Brevet francais (B.S.M.) n° 3.532 M.*

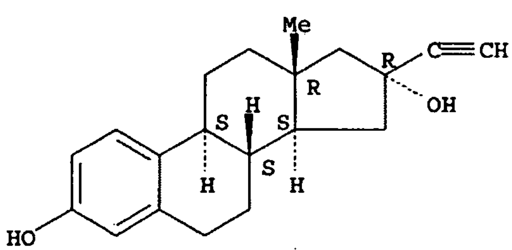
affinity and specificity of the natural hormones was determined Mols. interacting markedly with several receptors were submitted to x-ray crystallog. in order to establish whether overlap between the various conformations of the natural hormone and of the test mol. might not partly account for lack of specificity.

L6 ANSWER 3 OF 3 CAPLUS
ACCESSION NUMBER: 1970:3637 CAPLUS
DOCUMENT NUMBER: 72:3637
TITLE: 3,16α-Dihydroxy-16β-ethynylestra-1,3,5(10)triene for treating hypercholesterolemia
PATENT ASSIGNEE(S): Roussel-UCLAF
SOURCE: Fr. M., 3 pp.
CODEN: FMXXAJ
DOCUMENT TYPE: Patent
LANGUAGE: French
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
FR 5099		19670626	FR	19660112

IT **24989-47-7P**
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(hypocholesterolemic effect of)
RN 24989-47-7 CAPLUS
CN Estra-1,3,5(10)-triene-3,16-diol, 16-ethynyl-, (16α)- (9CI) (CA INDEX NAME)

Absolute stereochemistry.



GI For diagram(s), see printed CA Issue.
AB The title compound (I) has a hypocholesterolemic effect and very low estrogenic activity. Thus, a mixture of 260 ml tert-amyl alc. and 90 ml C_6H_6 was heated to 60°, 23.5 g K added slowly, the mixture stirred 2 hr at 65°, 100 ml dioxane added at room temperature, C_2H_2 passed into the mixture for 2.5 hr, a solution of 3.9 g 3-hydroxy-16-oxoestra-1,3,5(10)-triene in 120 ml dioxane added, the mixture stirred 4 hr at 32-3° in the presence of C_2H_2 stream, cooled, excess saturated NH_4Cl added, the mixture extracted with C_6H_6, and the organic phase worked up to give 528 mg I, m. 215°, $[\alpha]_D$ 47.5° (c 0.7, dioxane). Crude I was purified by SiO_2 and Al_2O_3 chromatog. The daily dose for treating hypercholesterolemia is 5 mg/kg (female rat).